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THE ACUTE INHALATION TOXICITY OF PYROLYSIS PRODUCTS OF HALON 1301
SUBTITLE: KINETIC COURSE OF LUNG INJURY, DEGRADATION IN WORK PERFORMANCE, AND EXERCISE POTENTIATION OF LUNG INJURY AFTER PHOSGENE EXPOSURE

FINAL REPORT

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INTRODUCTION

This final report summarizes investigations that have focused on the lower respiratory tract toxicity of inhaled phosgene ($COCl_2$), and it describes how the injurious effects of this compound may be enhanced by post-exposure exercise and/or result in a reduction in work performance capacity. Additionally, information is provided as to how increases in minute ventilation during exposure to phosgene affects the severity of the injurios response.

The major objectives of the project were:

Objective 1: To characterize the kinetics of onset and magnitude of lung injury following acute exposures to differing concentrations of phosgene delivered to rats over ten minute durations, using lung gravimetric, lung histopathologic, and lavage fluid constituent alterations as endpoints.

Objective 2: To assess how the ventilatory functions of rats are altered during acute exposures to phosgene.

Objective 3: To determine how kinetics of onset and magnitude of lung injury following acute exposures to phosgene may be altered when phosgene is inhaled under conditions of increased minute ventilation.

Objective 3: To determine how post-exposure exercise performed prior to and after the development of overt pulmonary edema may alter the course of development and severity of phosgene-induced lung injuy.

Objective 4: To assess how work performance, as indexed by maximum oxygen consumption by exercising rats, may be compromised following phosgene exposure.

Objective 5: To evaluate potential relationships between the magnitude of phosgene-induced lung injury and work performance incapacitation.

MATERIALS and METHODS

Animal Use and Welfare: Adult, male Fischer-344 rats weighing between 250 and 280 g were used in these studies. The stock from which the animals were derived was categorized as "Specific-Pathogen-Free, Virus-Free" by the supplier (Harlan Sprague Dawley, Inc., Indianapolis, IN). Upon arrival to Los Alamos, the rats were housed two per cage in polycarbonate cages covered with spun polyester filters (DuPont #22 Spinbound Polyester Filter, E.E. DuPont Co., Wilmington, DE) in an animal facility approved by the American Association for Accreditation of Laboratory Animal Care. The cages were maintained in air conditioned rooms that receive filtered air. Water and standard laboratory rat chow (autoclaved) were provided *ad libitum*. Prior to entry into any experimental study, the rats were maintained for a 2 week period in order to acclimate them to the laboratory facility, as well as to observe them for evidence of disease. In this latter regard, representative animals randomly selected from each shipment group were sacrificed with lethal intraperitoneal injections of pentobarbital sodium, blood serum was obtained for antibody titer levels, and their lungs were examined for disease. Animal sera were tested by Microbiological Associates (Bethesda, MD) for Reo 3, GDVII, KRV, H-1, M.AD, LCM, PVM, Sendai, and RCA. All sera tested over the last several years have been negative for the above infections, including sera obtained from animals maintained in our facility for over 3 months. Of relevance to the project, the body weights of the animals used in a given set of studies involving air and phosgene exposures were closely matched so their baseline lung gravimetric values would be virtually identical at the time of exposure (15, 33).

The Fischer-344 rat was selected as the animal model for the proposed studies for several reasons. First, the Fischer-344 rat is now widely accepted as a standard animal model for toxicological studies. Second, the use of a small animal model such as the rat allows studies to be performed with sufficient animal numbers for suitable statistical analyses. Third, the responses in the respiratory tracts of the rat and the human are similar following exposure to a variety of toxic substances. Lastly, the Fischer-344 rat has been used to assess the pulmonary toxicities of numerous other inhaled materials in our laboratory, including nitrogen dioxide, nitric oxide, hydrogen fluoride, hydrogen chloride, hydrogen bromide, bis(trifluoromethyl)disulfide, and perfluoroisobutylene (e.g., 1-16). Thus, toxicological data obtained from this phosgene study can be directly compared to information obtained about these other materials.

The protocols employed in this study were reviewed and approved by the Los Alamos National Laboratory Animal Care and Use Committee. We also note that the conduct and reporting of these studies have been and are in accordance with the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Council (DHEW Publication No. [NIH] 85-23,1985).

General Overview of Objectives and Approaches: Preliminary "range finding" studies were undertaken to assess the inhaled concentration-response characteristics of lung injury that occur as of 24 hrs after exposing groups of rats to differing concentrations of phosgene. In these studies, rats were exposed to either filtered air, 9.3 ppm, 13.9 ppm. 18.6 ppm, 23.3 ppm, 27.8 ppm, and 32.4 ppm for a duration of 10 min, Table 1. These animals were then sacrificed 24 hrs after the exposures and their lungs were assessed for weight changes and prepared for histopathologic analyses.

Thereafter, further studies of the kinetics of phosgene-induced lung injury were performed with the goal being to characterize the existence and duration of possible post-exposure latency periods, as well as to further assess the severity of phosgene-induce lung injury due to the inhalation of different phosgene concentrations. In these investigations, rats were exposed to either control air, or 9.3, 13.9, 18.6, or 23.3 ppm phosgene for 10 min. Lung injury was assessed at various times after exposure, as indicated in Table 1, using lung gravimetric, histopathologic, and, in selected experiments, lavage constituent alterations as endpoints (to be described).

To determine if the duration of a post-exposure latency period could be modified by increasing minute ventilation during phosgene exposure, rats were exposed to either 9.3 ppm or 13.9 ppm phosgene with and without concurrent CO_2 inhalation. Animals were then sacrificed at various times after exposure to characterize the times to onset of lung injury.

For the post- exposure work performance characterization and exercise potentiation components of the study, animals were exposed to either 9.3 ppm or 13.9 ppm phosgene for 10 min and exercised at various times after exposure, Table 2, and they were sacrificed either shortly after exercising or after a more extended period of time, depending on the experimental question being addressed. Corresponding control groups consisted of rats that were exposed to air only and exercised or rested until sacrifice, and phosgene-exposed animals that were allowed to rest prior to sacrifice. During the exercise bouts metabolic data were measured (to be described).

PHOSGENE EXPOSURE MATRIX	
PHOSGENE CONCENTRATION-RESPONSE STUDIES	
PHOSGENE Nominal Exposure Concentration	**Post-Exposure Sacrifice Times (Hr)**
9.3 ppm	24
13.9 ppm	24
18.6 ppm	24
23.3 ppm	24
27.8 ppm	24
32.4 ppm	24
PHOSGENE KINETIC STUDIES	
PHOSGENE Nominal Exposure Concentration	Post-Exposure Sacrifice Times (Hr)
9.3 ppm	1,3,5,6,7,8,10,18,24
13.9 ppm	1,3,6,24
18.6 ppm	1,2,3,4,6,24
23.3 ppm	1,3,24
PHOSGENE KINETIC STUDIES WITH INCREASED VENTILATION	
9.3 ppm + 5% CO_2	1,2,3,4,8,24
13.9 ppm + 5% CO_2	1,3,6,24
PHOSGENE LAVAGE STUDIES	
9.3 ppm	1,6,10,24
13.9 ppm	2,6,24

Table 1. Experimental exposure matrix for the Phosgene studies.

PHOSGENE EXPOSURE MATRIX		
PHOSGENE EXERCISE STUDIES		
Nominal Exposure Concentration	Post-Exposure Exercise Times	Post-Exposure Sacrifice Times (Hr)
9.3 ppm	0	0.5
9.3 ppm	4	5
9.3 ppm	5	6
9.3 ppm	6	7
9.3 ppm	7	8
9.3 ppm	9	10
9.3 ppm	17	18
9.3 ppm	23	24
9.3 ppm	0	24
13.9 ppm	2	3
13.9 ppm	5	6
13.9 ppm	23	24

Table 2. Experimental exposure matrix for the PFIB studies.

Atmosphere Generation and Characterization: An exposure system for delivering phosgene to laboratory rats with capabilities for measuring the ventilatory responses of rats before, during, and after exposure was designed and fabricated, Figure 1. All operations for the storage of phosgene, atmosphere generation, and atmosphere scrubbing occur within a Labconco Carcinogen/Toxic Gas Glove Box maintained at -2.5 cm H_2O pressure. The glovebox has internal HEPA filters on the inlet and exhaust as well as activated charcoal filters (3.5 lbs) on the exhaust. An internal blower maintains a glovebox airflow rate of 50 cfm. Laboratory rats were individually exposed to test atmospheres using an exposure system similar to that which was recently designed and fabricated for pulmonary toxicologic investigations of perfluoroisobutylene and bis(trifluoromethyl)disulfide (1, 12). The exposure atmospheres were contained within an exposure system, Figure 1, which included an atmosphere generator, delivery tubing and valves, an animal exposure tube, and charcoal absorbing bed. Exposure system materials were composed of Teflon® (TFE) or stainless steel (SS). Exposure atmospheres of up to 32 ppm were generated and delivered to rats for durations of 10 min. Phosgene (4000 ppm, Matheson Gas, LaPorte, TX) in nitrogen was stored within a storage cylinder at 1500 p.s.i.g. inside the glovebox. A gas delivery manifold attached to the storage cylinder consisted of a SS mass flow control valve and a normally closed solenoid valve. The solenoid valve was included into the system for safety considerations. The mass flow controller valve was used to deliver phosgene gas directly into a pure HEPA-filtered, dry diluting air stream (3 $L \cdot min^{-1}$). The exposure atmosphere was passed through a valve (V1) before being delivered to the experimental animal. Valve V1 in Figure 1 is a large bore TFE 3-way valve, and, when actuated with another valve (V2), it provided a means for the exposure atmosphere to bypass the animal exposure tube while simultaneously delivering clean air to the animal. This arrangement allowed for the safe installation and removal of the animal exposure tube, as described later. Valve V2, a flow controlled valve with solenoid ON-OFF features, was connected to clean HEPA filtered air. Downstream from the animal exposure tube and bypass circuit, an ASC Whetterite charcoal (VWR Scientific, Los Alamos, NM) absorption tower captured the exposure atmosphere. The exposure system was designed to work at a slightly positive pressure (< 0.1 cm H_2O) relative to the glovebox; by design, it was incapable of over-pressurization.

Measurement of the exposure atmosphere by gas chromatography (GC) occurred from ports located prior to the animal holding tube, after the animal holding tube, and at the exhaust of the charcoal filter stack. A compact, dedicated, dual column GC has been built

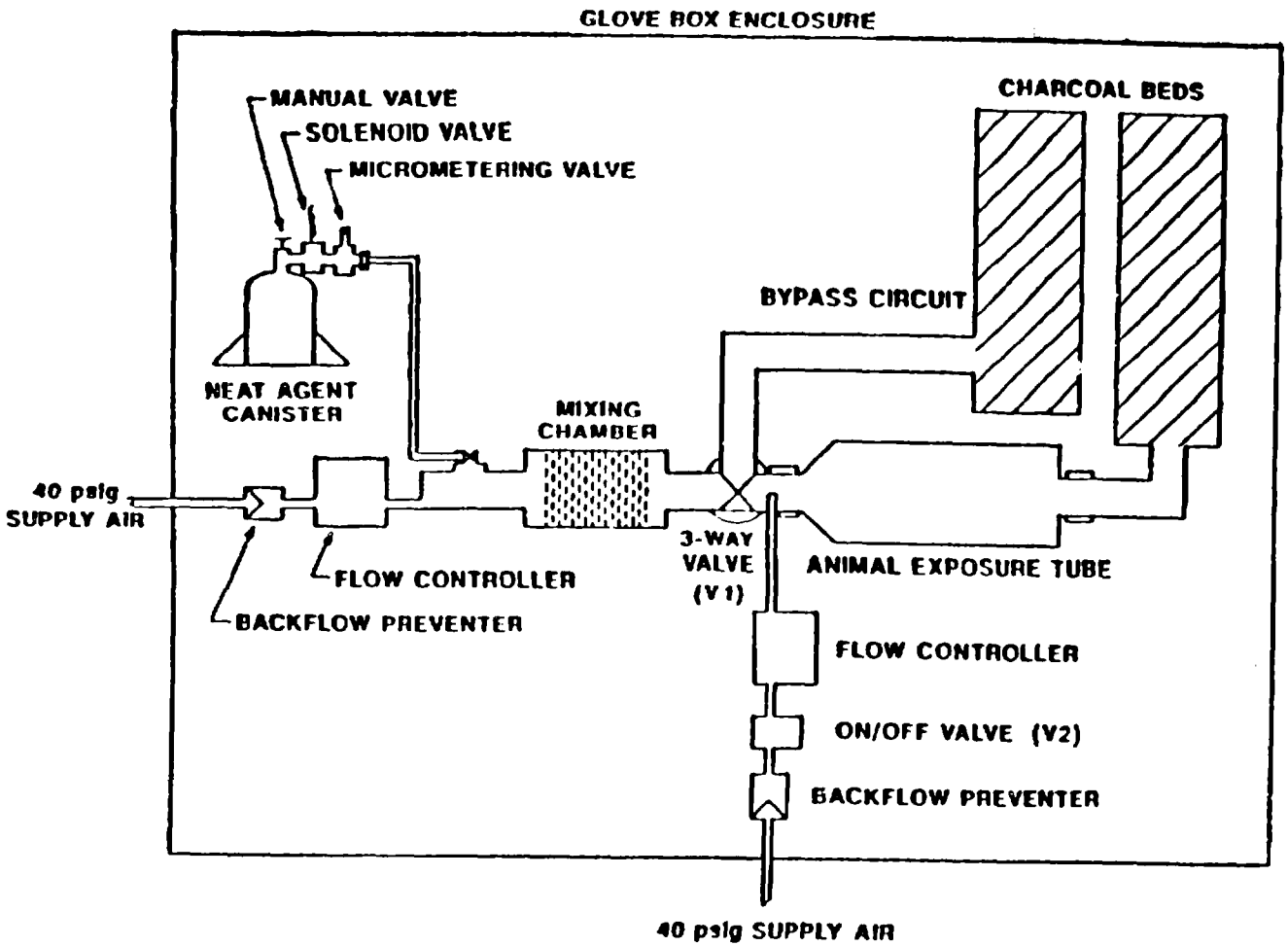

Figure 1: Schematic diagram of the exposure system to be used for the air and phosgene exposures.

for monitoring halogenated compounds in air. The detector for the GC peaks is an electron capture detector (Model 140B, Valco Instruments, Houston, TX) for maximum sensitivity. The carrier gas, argon/methane (90:10), was passed through a heated catalytic purifier (Supelco, Inc., Bellefonte, PA) to remove oxygen and water. Column and sample loop purge flows (10-20 $cm^3 \cdot min^{-1}$) were controlled by pressure regulators and needle valves. Sample loops and chromatographic columns (0.5 M and 1 M x 3 mm O.D. TFE columns containing 80/100 mesh Chromosil 310 (Supelco, Inc.) were attached directly to electrically actuated valves. Output from the electron capture amplifier was sent to a chart recorder/chromatographic peak integrator (Spectra Physics, SP 4270, San Jose, CA). A valve sequence programmer (Valco Instruments) and solenoid valves were used to automatically initiate sampling and analysis at periodic intervals while venting the interfering peak of oxygen. Air was drawn continuously through sampling loops except when it was being briefly directed to the GC columns. Careful adjustment of column lengths, carrier flows, and temperature allowed overlapping chromatograms from simultaneous sampling at two points. Sensitivities of this system for phosgene was ~1 ppb. The gas chromatograph was routinely calibrated with known dilutions of neat material. Once an atmosphere had been established and calibrated, it was diverted into the exposure by-pass circuit. The animals were then sealed into the exposure tubes and passed through the glovebox air-lock. The exposure tubes were secured onto the exposure system with swagelock type fittings. An exposure atmosphere was then directed to the animal for the 10 min exposure. After exposure, the atmosphere was again diverted from the exposure tube, and the animal exposure tube was flushed with clean, fresh air prior to animal removal.

Measurement of Ventilatory Parameters: Measurements of ventilatory function of unanesthetized rats took place before, during, and after exposure to air or phosgene by using partial body-flow plethysmographs, Figure 2. The plethysmographs were fabricated from TFE, and they consisted of nose sections (through which test atmospheres flow), head sections, body sections, and flow resistance plugs. The nose section allowed for the attachment of the plethysmograph to the exposure system manifold. The head section contained rubber dam diaphragms that effectively sealed the body of the rat into the plethysmograph. The head portion also contained a neck brace to restrain the head of the rat into the seals. The body section of the plethysmograph contained the body of the rat, and it attached to the nose section and the flow resistance plug via O-rings. The flow resistance plug contained a port covered with 4 layers of 400 mesh SS cloth, which provided a resistance to flow created by the expansion and contraction of the thorax within

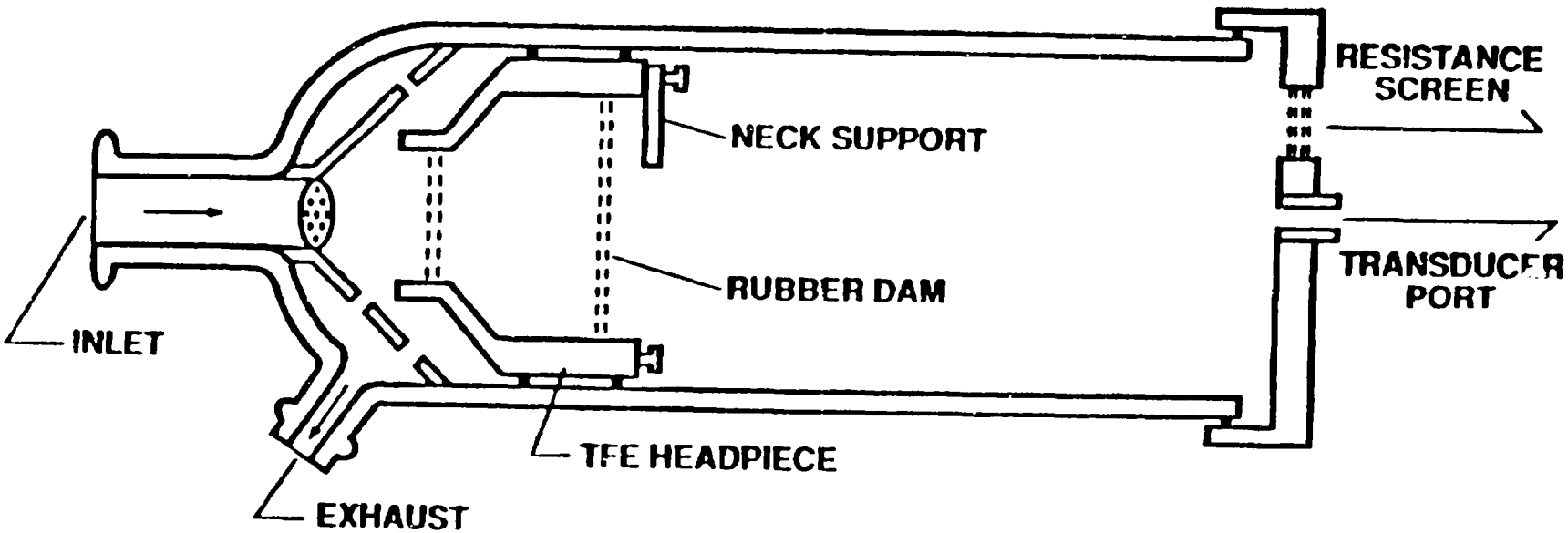

Figure 2: Schematic diagram of an exposure tube that also serves as a partial body flow plethysmograph.

the body section of the plethysmograph. Another port led to a differential pressure transducer (Validyne, Eng., ± 2.0 cm H_2O). The transducer was activated via a carrier demodulator (Validyne, Eng.), and its output was fed to a Gould strip chart recorder (Model No. 3009-115X, Gould, Cleveland, OH) and automated data acquisition and analysis system. The data acquisition and analysis system, developed at Los Alamos in collaboration with the U.S Army Walter Reed Army Institute of Respiratory Research, includes an IBM PC running ASYST software tools and custom software. The system is able to continuously measure ventilatory patterns from up to 6 rats within separate plethysmographs, and it converts these signals to breath by breath measurements of minute ventilation (V_E), breathing frequency (f), tidal volume (V_t), inspiratory time (T_I), and expiratory time (T_E). The system can also perform statistical analyses of these patterns. Similar body plethysmographic exposure systems have been used in our laboratory in other U.S. Army-funded investigations of the toxicities of halides, nitrogen dioxide, perfluoroisobutylene, and bis(trifluoromethyl)disulfide in the respiratory tract and in the U.S. Army-supported development of a cross-ventilating rat model (e.g., 8, 17).

Pre-exposure ventilation measurements were acquired for a minimum period of 10 min, and pulmonary ventilation values were measured continuously during the exposures. After an exposure, the atmosphere was diverted from the exposure tube, and the animal exposure tube was flushed with clean, fresh air while measurements of the ventilatory parameters continued to be measured prior to animal removal.

Maximum Oxygen Consumption (VO_{2max}) Measurements: Before use in the work performance or exercise components of this study, rats were subjected to a 20 day training program designed to behaviorally and physically condition them to perform on a treadmill, as described elsewhere (15). During the training program, the work intensities and durations of exercise were increased daily until the rats were capable of performing a "ramp" exercise protocol, Figure 3. Animals that were observed to be "non-runners" during the training sessions were eliminated from the exercise studies. The "ramp" protocol began at a treadmill velocity of 10 $M \cdot min^{-1}$. Every 30 sec thereafter, the treadmill velocity was incrementally increased by 5 $M \cdot min^{-1}$ up to a final velocity of 60 $M \cdot min^{-1}$ (15% grade). Maintenance of running speed was encouraged by electro-shock stimulation (40V, 2 mA) delivered via a grid (Coulburn, Lehigh, PA) mounted behind the treadmill. Prior to the "ramp" runs, the rats performed a "familiarization run" consisting of two short runs (20 $M \cdot min^{-1}$ for 3 min, 15% grade) separated by a 3 min rest period and finally followed by a 10 min rest period before initiation of the actual "ramp" protocol.

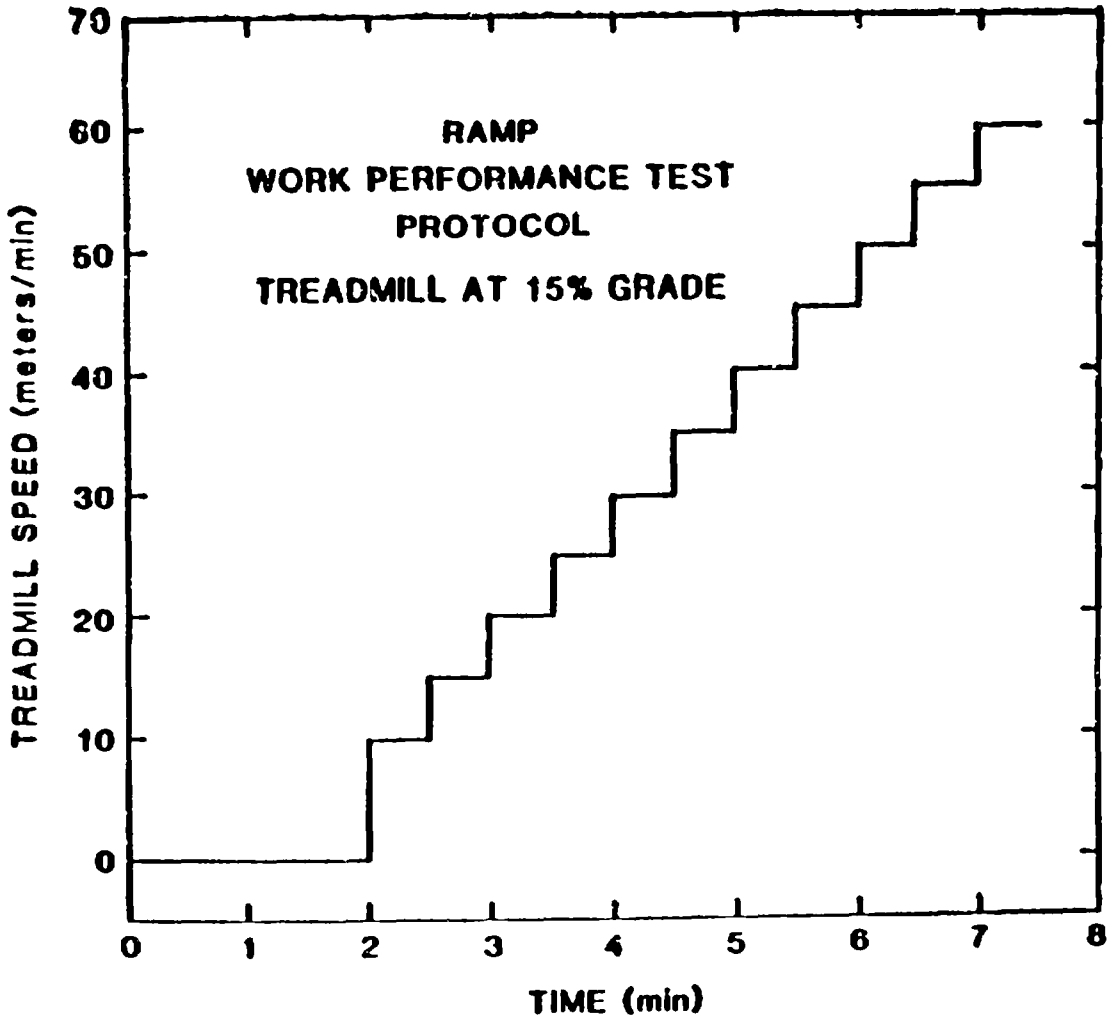

Figure 3: "Ramp" exercise protocol used to examine the post-exposure potentiating effects of exercise on phosgene-induced lung injury and for measuring maximum oxygen consumption (VO_{2max}).

Subsequent reference to "exercise" in the work performance and exercise potentiation components of this study includes the "familiarization run".

Maximum oxygen consumption (VO_{2max}) attained during performance of the "ramp" protocol was measured as a metabolic index of work performance incapacitation because of its close association with endurance (18). The treadmill used for the "ramp" protocol was contained in a metabolic chamber that provides the necessary means to measure oxygen consumption and carbon dioxide production as a rat exercises (3, 19), Figure 4. Airflow from the chamber (14 $L \cdot min^{-1}$) was dried (Silica Gel, J.T. Baker Chemical Co., Phillipsburg, NJ) and measured electronically using a pneumotachograph (Fleish No. 0, Gould Inc., Cleveland, OH) and a transducer (Validyne Engineering, Northridge, CA) that was calibrated spirometrically. Oxygen and carbon dioxide concentrations in the effluent airstream were measured with suitable analyzers (Ametek S-3A O_2 Analyzer, Ametek CD3A CO_2 Analyzer, Ametek, Pittsburg, PA) that were calibrated with primary gas standards (Matheson Gas, LaPorte, TX). Oxygen consumption and carbon dioxide production were calculated every 3 sec via a data acquisition and computer system (HP-3497A, Hewlett-Packard, Corvallis, OR) using the equations of Mautz et al. (20). Maximum oxygen consumption, defined as the plateau of oxygen consumption achieved with increasing work loads, was expressed as ml O_2/kg body weight/min following correction for standard temperature and pressure. As illustrated in Figure 5, the VO_{2max} values of animals performing the "ramp" protocol become satisfactorily stable and reproducible during the 20 day training program. As also indicated in Figure 5, trained rats typically consume oxygen maximally within ~5-6 min after the "ramp" protocol is initiated.

Animal Sacrifices, Lung Gravimetric Measurements, and Histopathology: Animals were deeply anesthetized with I.P. injections of 50 mg pentobarbital sodium. After exsanguination via ligation of the jugular veins and carotid arteries, the trachea and lungs were excised, and the heart, extrapulmonary mediastinal tissue, and the esophagus were removed. The lung was blotted dry and weighed (Lung Wet Weight, LWW). The bronchus leading to the right cranial lobe (RCL) was ligated with fine suture and the RCL was removed, weighed (Right Cranial Lobe Wet Weight, RCLWW), and subsequently dried in an oven at 100°C for 48 hrs and reweighed (Right Cranial Lobe Dry Weight, RCLDW). Percent changes in the lung gravimetric parameters described herein were calculated as: [(post-exposure mean value - air control mean value/air control mean value)·100%]. Inasmuch as post-exposure changes in the RCLDW and LWW endpoints

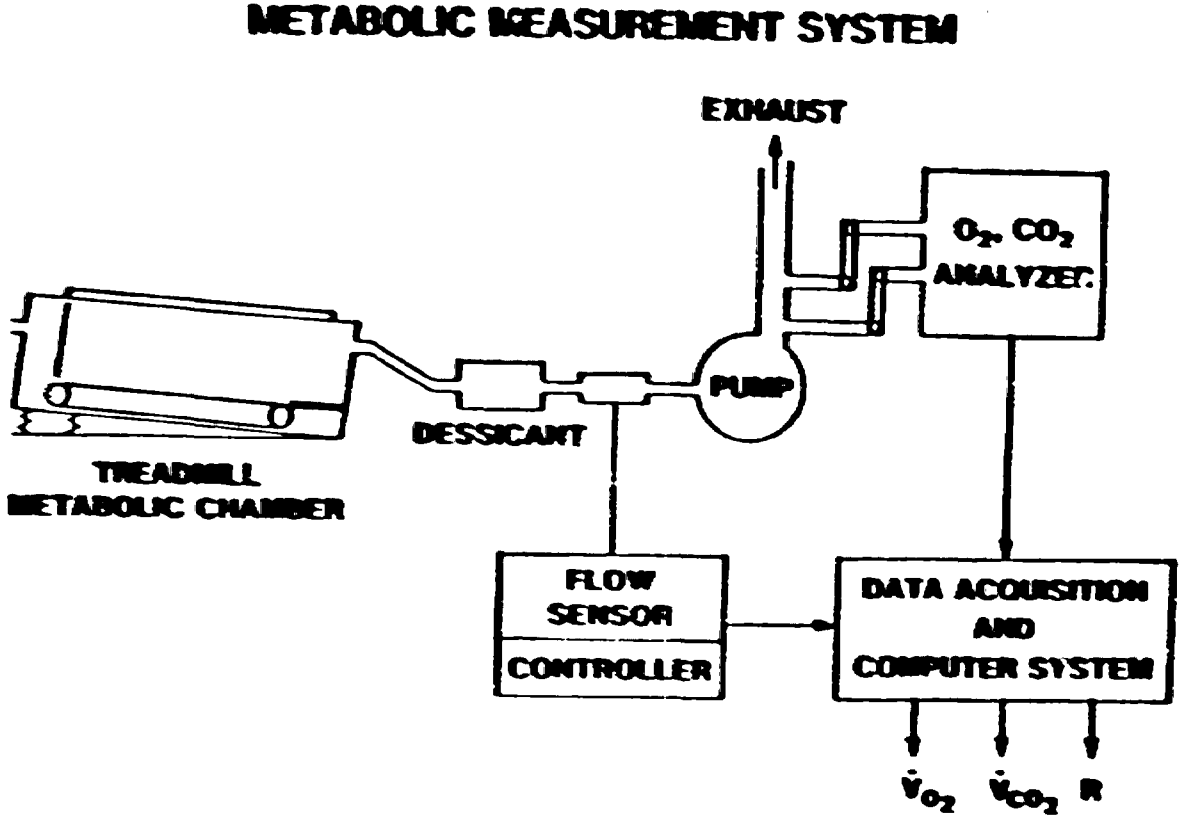

Figure 4: Schematic representation of the treadmill-metabolic chamber system used for measuring $\dot{V}O_{2max}$.

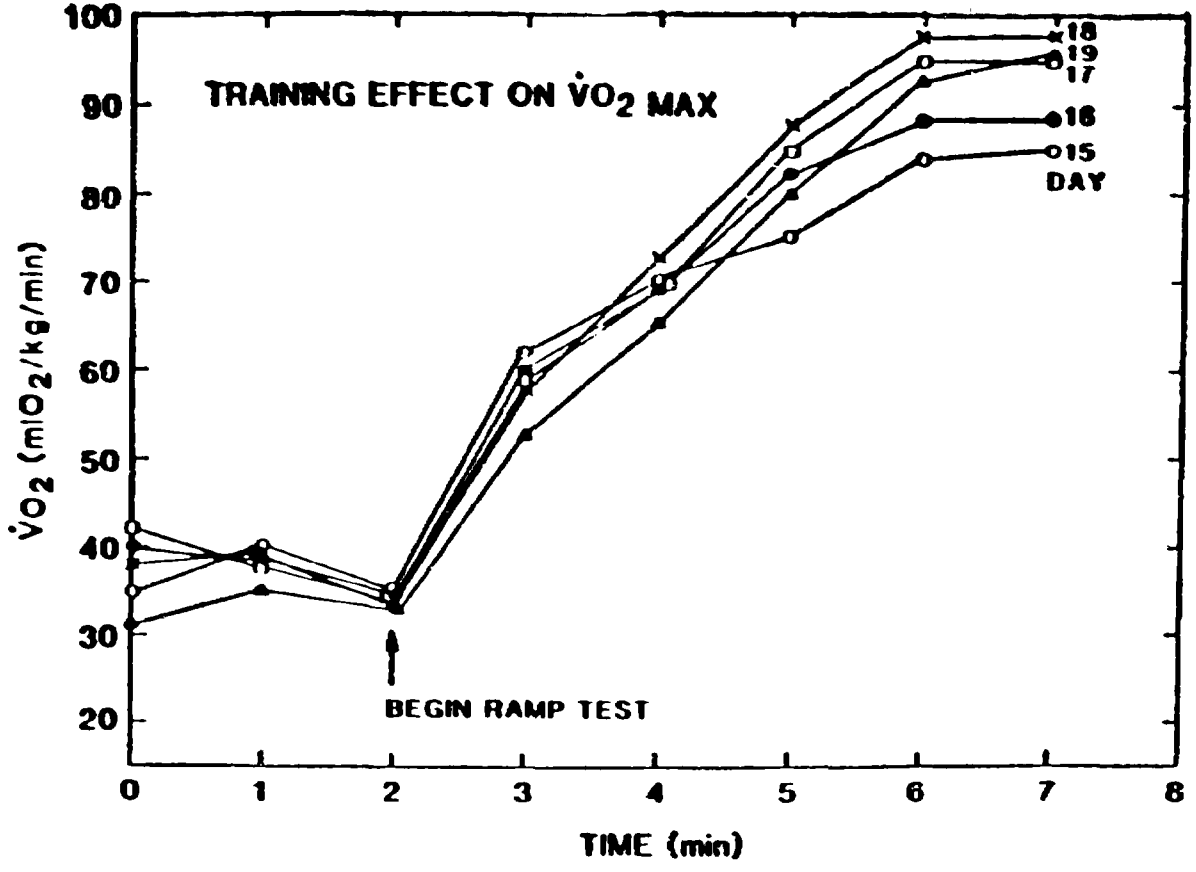

Figure 5: Oxygen consumption and $\dot{V}O_{2max}$ of rats during the last several days of the 20 day training protocol.

generally paralleled one another, only the LWW and RCLDW data are presented in this report.

The lungs (minus the RCL) were routinely filled at a pressure of 30 cm H_2O with 10% formalin in phosphate-buffered saline for 48 hrs. For histological analysis of the lung, each left lobe was sliced on the same plane as the main-stem bronchi from its apex to its base along a line between the most posterior to the most anterior aspects to expose the maximal planar surface area for sectioning (1, 4, 8, 13, 15, 16). The blocks of tissue were embedded in paraffin, and 4 μm sections were prepared and stained with hematoxylin and eosin by conventional methods.

Semi-Quantitative Histopathology: Histopathologic assessments focused on the appearance of edema fluid and fibrin, abnormal accumulations of polymorphonuclear leukocytes (PMN) and alveolar macrophages (AM), vascular congestion, and alveolar cuboidal cell hyperplasia, i.e. Type II cell hyperplasia (22) With the exception of vascular congestion, a grading scale was used to quantitatively describe the relative extent of each of the above pathologic features in terms of their: 1) distribution, i.e., relative portion of the lung showing a lesion; 2) severity, or the relative number of alveolar structures affected within the lesion area; and 3) intensity, i.e., the relative amount of material or relative alterations of cells in the affected alveoli. The distribution index for a given pathologic feature ranged from 0-4 with 0 = not observed, 1 = single or focal in appearance, 2 = few but multifocal, 3 = moderate portion of the lung involved, and 4 = all or essentially all alveolar structures were affected, i.e., diffuse. The relative severity index for a given pathologic feature ranged from 0 to 4 with 0 = no abnormality, 1 = the focal appearance of the abnormality in the lesion area alveolar structures, 2 = focal to multifocal appearance in the lesion area alveolar structures, 3 = several affected alveolar structures, and 4 = many to all lesion area alveolar structures showed the abnormality. The relative intensity index also ranged from 0 to 4 with 0 = no abnormality, 1 = trace, but detectable alterations in the amount of abnormal material, 2 = mild amount of material or small changes in cell numbers, 3 = moderate amount of abnormal material or abnormal number of cells, and 4 = large amounts of intraalveolar material or large changes in cell numbers. For grading vascular congestion, only the distribution and severity indices were used. In this case, the distribution index refers to the relative numbers of alveolar structures that showed capillary congestion in associated alveolar septal walls, and the severity index references the relative numbers of alveoli involved. The above histopathologic examinations were performed in a statistically blind fashion. It should also be noted that the above scoring sytem is similar to

that used in previous studies in our laboratory in which the injurious effects of other toxic gases have been characterized (e.g., 1, 8, 13, 15).

Bronchoalveolar Lavage and Lavage Fluid Processing: At designated post-exposure times, rats were anesthetized with intraperitoneal injections of pentabarbital sodium (50 mg). Prior to complete apnea, the rats were exsanguinated via carotid artery transection, and their tracheae were fitted with 18 ga. blunt needles that were secured with ligature. The lungs and trachea of each animal was excised from the thoracic cavity *en bloc*, and the heart and esophagus was removed. The lavage protocol consisted of six sequential lung wash cycles using 6 ml of room temperature, divalent cation-free phosphate buffered saline (PBS, made with HPLC grade H_2O) per wash (21). The lung washings were performed by gently massaging the lungs during the infusion and aspiration of the PBS during each cycle. Lavage fluid retrieved from each lung was pooled in a centrifuge tube maintained in ice. On average, ~85% of the instilled lavage fluid was recovered from the lungs of air and phosgene exposed animals.

Prior to centrifugation of lavage fluids, small aliquots of the recovered lavagates were used for cell counts, for assessments of cell viability, and for preparing cell cytocentrifuged (Shandon Southern Cytospin, Shannon Southern Products, Ltd., Chesire, UK) slide preparations. Cell counts were performed with a hemocytometer. Cell differential analyses of each cytocentrifuged lung free cell population were performed by evaluating a minimum of 300 cells as to cell type after the cells were stained with Diff Quik® (American Scientific Products, McGaw Park, IL).

The lavage fluid was centrifuged at 300 g for 10 min at 4°C to sediment the lung free cells. The supernatant above each cell pellet was aspirated, placed in another centrifuge tube, and centrifuged at 2300 g for 10 min at 4°C to sediment any remaining acellular, insoluble material (9, 14). Supernates that were obtained following this last centrifugation cycle are referred to herein as bronchoalveolar lavage fluids (BALF).

Aliquots of BALF were used to measure lactate dehydrogenase (LDH) (Sigma Chemical Co.: Lactate Dehydrogenase Kit, NADH Cat. No. 340-101, Pyruvate substrate, Cat. No. 500L-1, Color Reagent, Cat No. 505-2). LDH is a cytoplasmic enzyme present in all cells, and it is released extracellularly when cells undergo lysis or when cell membrane permeability is increased due to damage (3, 23, 24). Enzymatic activities were expressed in International Units (micromoles of substrate converted per min) on an ml basis. After obtaining the aliquots of BALF for the LDH assays, the BALF were stored in polypropylene tubes at -70°C until further analyzed. Aliquots of thawed BALF were analyzed for protein using the method of Lowry et al. (25) and bovine serum albumin as a

protein standard. This biochemical parameter was used as an index of the amount of transudation of serum proteins into the alveoli because of damage to the alveolar-capillary barrier (26, 27). As previously reported by others (28), abnormal increases in protein in lavage fluid following phosgene exposure has been shown to be a more sensitive indicator of lung damage than are lung gravimetric increases and lung histopathologic changes. Results from the protein assays are expressed as absolute mass of protein measured on an ml basis.

Because a general protein assay such as the Folin-Lowry assay is also sensitive to some non-protein biochemicals, e.g., hydrolysis products of nucleic acids that may be present due to cell death, (29), and because other sources of abnormal increases of protein in lavage fluid may include the excessive production of airway mucus (glycoproteins) in response to a toxic material and the release of proteins from lysed cells, further analyses of BALF were performed to confirm that the protein increases found after phosgene exposure were associated with increases in proteins derived from the blood and thus reflect permeability changes per se. In these analyses, some specific, blood-derived biochemical constituents in BALF, i.e., albumin and transferrin, were further resol.ed and quantitated using ion exchange high performance liquid chromatographic method (HPLC) (3, 30). The basic HPLC system consisted of a Waters Model 600E Controller (Waters Associates, Milford, MA) and an ion exchange column (IEC) (Protein-Pak DEAE-5PW column, 7.5 mm x 7.5 cm, Waters Associates). Three ml aliquots of thawed BALF were loaded onto the IEC, and constituents contained therein were eluted over a 30 min period at a flow rate of 1 $ml \cdot min^{-1}$ using a linear NaCl and pH gradient ranging from 20 mM Tris buffer (pH 8.5) to 20 mM Tris buffer containing 0.3 M NaCl (pH 7.0). Eluted lavage constituents were detected by uv absorbance at 280 nM using a Waters 484 tunable absorbance detector (Waters Associates), which was connected to a Waters Model 745B Data Module (Walters Associates). Standards were used to determine that two of the resolved fractions represent transferrin and albumin, respectively, and this was further verified by collecting these fractions and reanalyzing them by reversed phase HPLC, as described elsewhere (9). The standards used in our analyses consisted of rat albumin (Organon Treknika Corp., West Chester, PA) and rat transferrin (Organon Treknika Corp.) prepared in PBS. The albumin and transferrin fractions were quantified in the post-exposure studies by measuring the areas under their resolved peaks.

Statistical Analyses: Body weight and lung gravimetric data were analyzed for significant differences between groups using a one-tailed t-test (31). The selection of a one-tailed t-test for these parameters was based on our previous investigations that have

demonstrated that environmental insults essentially invariably result in decreases in body weights and increases in lung weights when the environmental insults cause acute lung injury. The histopathologic data were compared using the Mann-Whitney nonparametric test for unpaired data (32). The Mann-Whitney test was selected because the numeric expressions of the various histopathologic features of toxic gas-induced lung injury, as indexed by the previously described semi-quantitative scoring system, often do not appear to follow a Gaussian distribution within a given exposure group. Statistical comparisons of lavage fluid constituent changes were made using two-tailed t tests (31). In all statistical comparisons, probability values (p) ≤ 0.05 were considered to denote significance differences.

RESULTS

Initial "Range Finding" Concentration-Response Studies

Body Weight Changes: Animals exposed to air only gained on average ~1% of their initial body weights 24 hrs after the exposure, Table 3. In contrast, animals exposed to all concentrations of phosgene studied lost from 3% to 6% of their body weights during the first 24 hr period after the exposure. Generally, the average loss in body weight following phosgene exposure increased with increasing phosgene exposure concentration.

Lung Gravimetric Changes: Concentration-response relationships of LWW and RCLDW values measured 24 hrs after exposure to various concentrations of phosgene are graphically summarized in Figures 6 and 7, respectively. Significant increases in both LWW and RCLDW were detected 24 hrs after exposure to phosgene concentrations equal to and greater than 9 ppm. As indicated in Figures 6 and 7, the magnitude of phosgene-induced increases in LWW and RCLDW increased with exposure concentration, with changes in LWW and RCLDW following virtually identical patterns as a function of inhaled phosgene concentration. Approximately 40% of the animals exposed to 32.4 ppm phosgene died within 24 hrs after the exposure (~LD_{40}). Thus, the 32.4 ppm phosgene LWW and RCLDW values shown in Figures 6 and 7 were obtained with surviving animals only.

Lung Histopathology: No histological abnormalities were observed in the lungs of rats exposed to air only (data not shown). Major histological evidence of damage

TIME POST EXPOSURE	EXPOSURE TREATMENT						
	AIR	Phosgene 9.3 ppm	Phosgene 13.9 ppm	Phosgene 18.6 ppm	Phosgene 23.3 ppm	Phosgene 27.8 ppm	Phosgene 32.4 ppm
24 HR	2	-8*	-15*	-18*	-20*	-25*	-18*

Table 3. Mean body weight change (gm) upon sacrifice after exposure to air or various nominal concentrations of Phosgene. (*) denotes significant difference from air control values ($p \leq 0.05$). Each exposure group consisted of 5 - 8 rats.

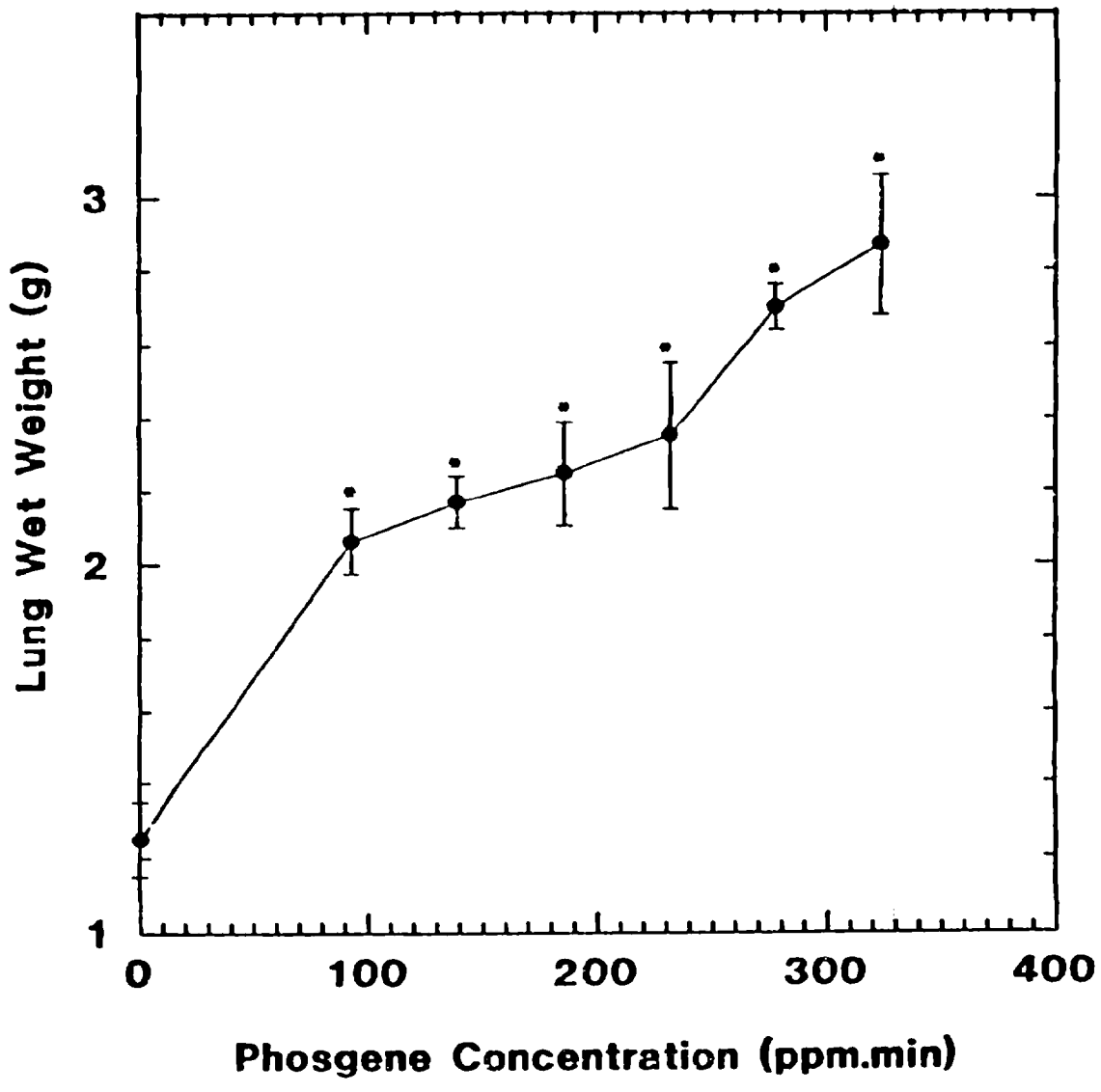

Figure 6. Lung Wet Weight (LWW) values measured on animals exposed to air or various nominal concentrations of Phosgene for a 10 minute duration. (*) indicates significant increases compared to values measured on animals exposed to air only, ($p \leq 0.05$).

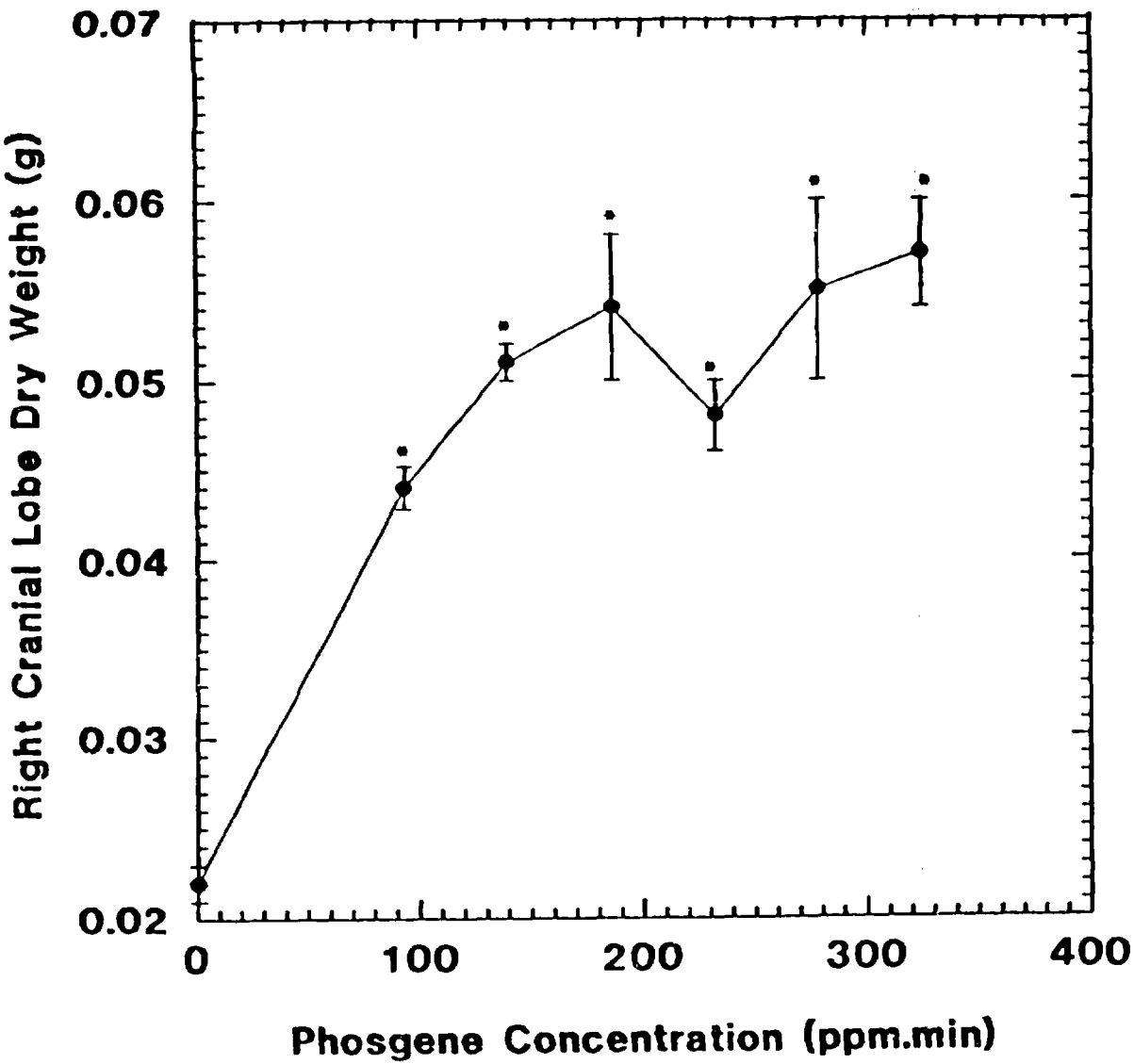

Figure 7. Right Cranial Lobe Dry Weight (RCLDW) values measured on animals exposed to air or various nominal concentrations of Phosgene for a 10 minute duration. (*) indicates significant increases compared to values measured on animals exposed to air only, ($p \leq 0.05$).

was detected in the lungs of animals 24 hrs after exposure to phosgene, regardless of the exposure mass concentration, Tables 4a and 4b. Histopathologic evaluation of the lungs of animals 24 hrs after exposure to various concentrations of phosgene revealed no differences of in lung pathology between the different concentrations. Significant increases in the distribution and amount of fibrin observed in the lung of animals exposed to all concentrations of phosgene was a hallmark of the lesion. Also significant increases in the distribution and abundance of PMN and AM were prominant features 24 hrs after exposure to all concentrations of phosgene examined. No evidence of perivascular congestion was observed following these exposure concentrations.

Phosgene Concentration-Kinetic Response Studies

Body Weight Changes: Initial loses of approximately 3% body weights occurred within the first 6 hrs after exposure to all experimental atmospheres, including air, Table 5. Weight losses were recovered by 24 hrs after exposure with animals exposed to air only, whereas body weights continued to be diminished in those animals exposed to all concentrations of phosgene. Such body weight losses generally increased with increasing exposure mass concentration of phosgene.

Lung Gravimetric Changes: LWW and RCLDW values measured at various times after exposure to the several different concentrations of phosgene are summarized in Figures 8 and 9, respectively. Specific changes that occurred are separately described below. It should be noted that for each phosgene concentration examined, a corresponding control group of animals was exposed to clean air only. It should also be noted that relative to unexposed, cage control rats, no significant differences occur in the LWW or RCLDW parameters when animals were exposed to clean, filtered air for 10 min and examined over a 48 hr post-exposure period.

Lung Gravimetric Changes after Exposure to 9.3 ppm Phosgene Exposure

No significant increases in LWW or RCLDW were detected 1, 3, 5, or 6 hrs after exposure to 9.3 ppm phosgene. However, compared to air exposed control values, significant increases in LWW of approximately 10%, 17%, 46% and 72% were observed at the 8, 10, 18 and 24 hr sacrifice time points, respectively, after exposure to the 9.3 ppm phosgene concentration. While RCLDW values were not significantly elevated as of 8 hrs after exposure, they were significantly elevated ~19%, and ~95% above air control values

Nominal Exposure Concentration ppm	*Distribution*	*Severity*	*Intensity*
	Edema Fluid		
9.3	0	0	0
13.9	0	0	0
18.6	0.7 ± 0.5	0.7 ± 0.4	0.7 ± 0.4
23.3	0.6 ± 0.6	0.8 ± 0.8	0.6 ± 0.6
27.8	0	0	0
32.4	0	0	0
	Fibrin		
9.3	4.0 ± 0.0*	2.0 ± 0.0*	1.8 ± 0.1*
13.9	4.0 ± 0.0*	2.0 ± 0.0*	2.0 ± 0.0*
18.6	4.0 ± 0.0*	2.2 ± 0.2*	2.0 ± 0.0*
23.3	4.0 ± 0.0*	2.3 ± 0.2*	2.7 ± 0.2*
27.8	4.0 ± 0.0*	2.5 ± 0.5*	2.5 ± 0.0*
32.4	4.0 ± 0.0*	2.1 ± 0.1*	2.7 ± 0.2*
	Polymorphonuclear Leukocytes		
9.3	3.8 ± 0.2*	2.0 ± 0.0*	2.0 ± 0.2*
13.9	4.0 ± 0.0*	2.0 ± 0.0*	2.0 ± 0.0*
18.6	3.0 ± 0.0*	2.0 ± 0.0*	1.8 ± 0.2*
23.3	2.4 ± 0.2*	1.8 ± 0.2*	1.8 ± 0.2*
27.8	3.5 ± 0.5*	2.0 ± 0.0*	1.5 ± 0.5*
32.4	4.0 ± 0.0*	2.0 ± 0.0*	2.0 ± 0.0*
	Macrophages		
9.3	3.4 ± 0.2*	2.0 ± 0.0*	1.1 ± 0.1*
13.9	3.8 ± 0.2*	2.0 ± 0.0*	2.0 ± 0.0*
18.6	4.0 ± 0.0*	2.3 ± 0.2*	2.0 ± 0.0*
23.3	3.4 ± 0.2*	2.0 ± 0.0*	1.4 ± 0.2*
27.8	3.5 ± 0.5*	2.0 ± 0.0*	1.0 ± 0.0*
32.4	3.5 ± 0.2*	2.0 ± 0.0*	1.3 ± 0.2*

Table 4a. Histopathologic evaluation of the dose response of the lung 24 hr after exposure to various nominal concentrations of Phosgene. (*) denotes significant difference from air exposed values, $p \leq 0.05$.

Nominal Exposure Concentration ppm	*Distribution*	*Severity*	*Intensity*
	Type II Cell Hyperplasia		
9.3	2.0 ± 0.5*	1.6 ± 0.4	0.8 ± 0.2
13.9	0.8 ± 0.5	0.7 ± 0.4	0.3 ± 0.2
18.6	2.2 ± 0.5*	1.5 ± 0.3*	1.2 ± 0.3*
23.3	0	0	0
27.8	0	0	0
32.4	0	0	0
	Perivascular Congestion		
9.3	0	0	
13.9	0	0	
18.6	0	0	
23.3	0	0	
27.8	0		
32.4	0	0	

Table 4b. Histopathologic evaluation of the dose response of the lung after exposure to various nominal concentrations of Phosgene. (*) denotes significant difference from air exposed values, $p \leq 0.05$.

TIME POST EXPOSURE	EXPOSURE TREATMENT						
	AIR	Phosgene 9.3 ppm	Phosgene 13.9 ppm	Phosgene 18.6 ppm	Phosgene 23.2 ppm	Phosgene 27.8 ppm	Phosgene 32.4 ppm
1 Hr	-3	-3	-5	-4	-7		
3 Hr	-4	-5	-7	-8	-8		
6 Hr	-9	-8	-10	-9			
24 Hr	2	-4*	-15*	-19*	-20*	-21*	-18*

TABLE 5. Mean body weight change (gm) upon sacrifice after exposure to air of various concentrations of Phosgene. (*) denotes significant difference from air control values ($p \leq 0.05$). Each group consisted of 4 to 6 rats.

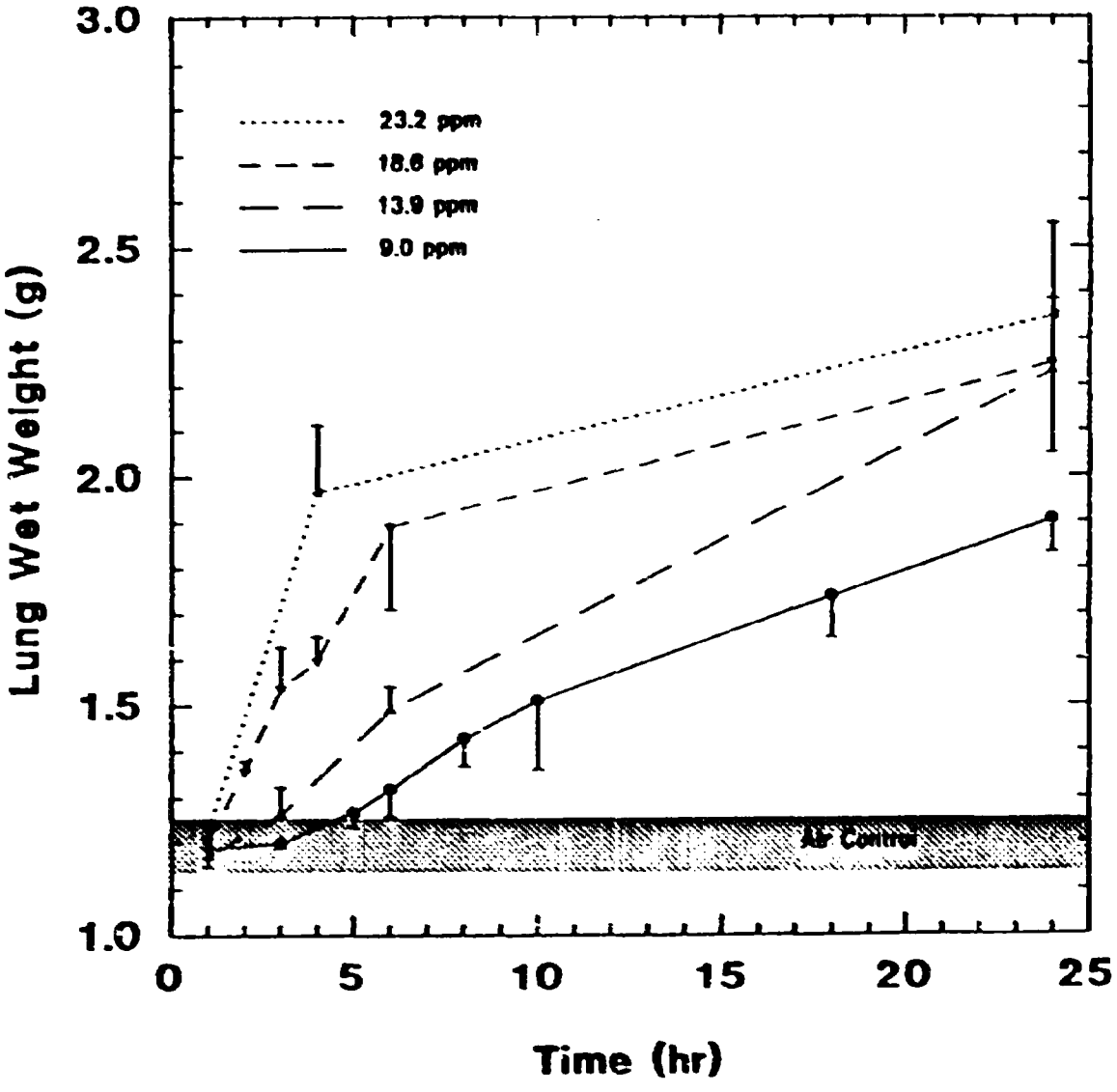

Figure 8. Kinetics of development of pulmonary edema as represented by increases in Lung Wet Weight (LWW) values measured at various times after exposure to several concentrations of Phosgene. Each point represents the mean and S.E.M. of N=5-8 rats. Error bars above the cross-hatch region indicate significantly greater responses, ($p \leq 0.05$), than values of LWW measured on animals exposed to air only.

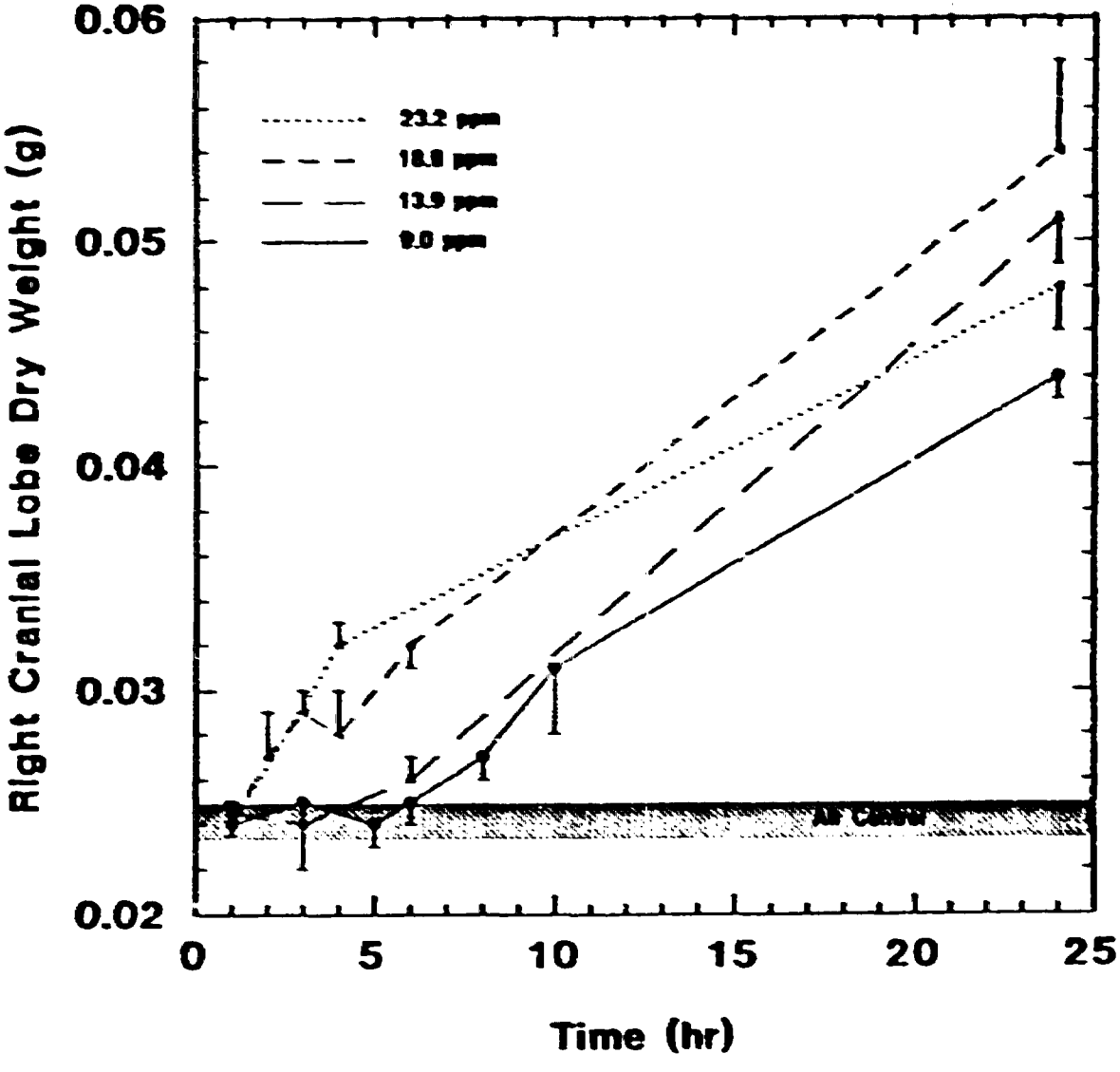

Figure 9. Kinetics of development of pulmonary edema as represented by increases in Right Cranial Lobe Dry Weight (RCLDW) values measured at various times after exposure to several concentrations of Phosgene. Each point represents the mean and S.E.M. of N=5-8 rats. Error bars above the cross-hatch region indicate significantly greater responses, ($p \leq 0.05$), than values of RCLDW measured on animals exposed to air only.

at the 10 and 24 hr post-exposure time points, respectively. RCLDW values were not collected for the 18 hr post phosgene exposure time point.

Lung Gravimetric Changes after Exposure to13.9 ppm Phosgene Exposure

LWW and RCLDW values at 1 or 3 hrs after exposure to 13.9 ppm phosgene were not significantly elevated relative to values obtained with control air exposed rats. LWW values significantly increased ~15% and ~83%, however, at 6 hrs and 24 hrs afterexposure to this phosgene concentration. RCLDW values were also elevated ~15% and ~131% at these time points, respectively.

Lung Gravimetric Changes after Exposure to18.6 ppm Phosgene Exposure

LWW · ·es measured 1 hr after exposure to 18.6 ppm were slightly, yet significantl· ıted (~6%) compared to values measured with animals exposed to air only. No .gnificant changes in RCLDW, however, were detected at this early post-exposure time point. More marked increases in LWW (~19%) and RCLDW (~13%) values were obtained as of 2 hrs after exposure to 18.6 ppm phosgene concentration. This rapid increase in LWW and RCLDW progressively continued at 3, 4, and 6 hrs after phosgene exposure, with LWW increases of ~19%,~24%, and ~47%, respectively, and RCLDW increases of ~12%, ~12%, and ~23%, respectively, above air exposed control values. At 24 hrs after 18.6 ppm phosgene exposure, LWW and RCLDW values were elevated ~89%, and ~145% respectively.

Lung Gravimetric Changes after Exposure to 23.2 ppm Phosgene Exposure

One hour after exposure to 23.2 ppm phosgene, slight but significant increases (~7%) in LWW were measured. No significant increases in RCLDW were found at this time point. As of 4 hrs after exposure to this phosgene concentration, more substantial increases in LWW (~52%) and RCLDW (~27%) occurred. Values for LWW and RCLDW were further increased (~96%) and (~109%), respectively, as of 24 hrs after exposure to the 23.2 ppm concentration of phosgene.

Lung Histopathology:

Changes after Exposure to 9.3 ppm Phosgene

The kinetic course of development of pathologic changes that occurred in the lungs of rats exposed to the 9.3 ppm concentration of phosgene is summarized in Table 6. Significant amounts of alveolar fibrin were present as early as 4 hrs after exposure. This

Exposure Concentration 9.3ppm	Distribution	Severity	Intensity
Sacrifice Time (hr)	Fibrin		
3	0	0	0
4	1.7± 0.6*	1.7 ± 0.3*	0.8 ± 0.2*
5	3.4 ± 0.4*	2.0 ± 0.0*	1.6 ± 0.2*
6	3.3 ± 0.3*	2.0± 0.0*	1.1± 0.1*
8	4.0 ± 0.0*	2.0 ± 0.0*	1.9± 0.1*
10	3.8 ± 0.2*	2.2± 0.2*	1.9± 0.2*
18	4.0± 0.0*	2.6 ± 0.1*	2.0± 0.0*
24	4.0 ± 0.0*	2.8 ± 0.2*	2.4 ± 0.2*
	Polymorphonuclear Leukocytes		
3	0	0	0
4	0	0	0
5	0	0	0
6	2.5 ± 0.3*	1.6 ± .3*	1.0 ± 0.0*
8	2.8 ± 0.3*	2.0 ± 0.0*	1.5 ± 0.2*
10	3.4 ± 0.2*	1.8 ± 0.1*	1.3 ± 0.1*
18	4.0 ± 0.0*	2.2 ± 0.1*	2.5 ± 0.3*
24	4.0 ± 0.0*	2.0 ± 0.0*	2.8 ± 0.2*
	Macrophages		
3	0	0	0
4	0.5 ± 0.3	0.7 ± 0.4	0.3 ± 0.2
5	2.2 ± 0.7*	1.4 ± 0.4*	0.8 ± 0.2*
6	2.5 ± 0.3*	1.8 ± 0.3*	1.3 ± 0.3*
8	3.8 ± 0.3*	2.0 ± 0.0*	1.3 ± 0.1*
10	3.8 ± 0.2*	2.0 ± 0.0*	1.4 ± 0.1*
18	4.0 ± 0.0*	2.0 ± 0.0*	1.0 ± 0.0*
24	3.8 ± 0.2*	1.8 ± 0.2*	1.0 ± 0.0*

Table 6: Histopathologic changes in the lung at various times after exposure to 9.3 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

abnormality progressively increased in severity and intensity up to 24 hrs after exposure, and by 18 hrs after exposure, alveolar fibrin was diffusely observed in the alveoli. Polymorphonuclear leukocytes (PMN) were observed as early as 6 hrs after exposure. Like the alveolar fibrin, the occurrence of these cells gradually became diffuse as their relative abundance increased. Significant increases in observable alveolar macrophages occurred as of 5 hr after exposure, and their excessive occurrence became generally diffuse thereafter. No evidence of extravasated erythrocytes or Type II cell hyperplasia was found in this study series.

Changes after Exposure to 13.9 ppm Phosgene

The kinetic course of development of pathologic changes that occurred in the lungs of rats exposed to the 13.9 ppm concentration of phosgene is summarized in Tables 7a and 7b. Similar to the results obtained afyer the 9.3 ppm exposure, exposure to 13.9 ppm phosgene caused significant increases in alveolar fibrin as of 3 hr after exposure, and the fibrin progressively increased in distribution, severity, and intensity thereafter. Also, the increases in the appearances of PMN and AM were similar to what occurred after the 9.3 ppm exposure. Unlike with the 9.3 ppm concentration, however, extravasated erythrocytes were a significant feature to the response to the 13.9 ppm concentration as of 24 hrs after exposure.

Changes after Exposure to 18.6 ppm Phosgene

The kinetic course of development of pathologic changes that occurred in the lungs of rats exposed to the ppm concentration of phosgene is summarized in Tables 8a and 8b. Most semi-quantitative values for alveolar fibrin, PMN, and AM were similar to those obtained at comparable time points with the lower concentrations of phosgene. Unlike with the lower concentrations, however, alveolar edema (which can be difficult to detect in lungs that have been fixed by the insufflation of the lungs with fixative), and Type II cell hyperplasia became significant components of the injurious response to the 18.6 ppm concentration of phosgene as of 24 hrs after exposure. Additionally, extravasated erythrocytes were also present 24 hrs after exposure.

Changes after Exposure to 23.2 ppm Phosgene

The course of development of pathologic changes that occurred in the lungs of rats exposed to the 23.3 ppm concentration of phosgene is summarized in Table 9. Whiie fibrin and PMN were an early component of the response to this concentration of phosgene, these and other histopathologic changes were not noted as of 24 hr after

Exposure Concentration 13.9 ppm	Distribution	Severity	Intensity
Sacrifice Time (hr)	**Fibrin**		
1	0	0	0
3	2.0 ± 0.0*	1.9 ± 0.1*	1.1± 0.1*
6	4.0 ± 0.0*	2.0 ± 0.0*	1.0 ± 0.0*
24	4.0 ± 0.0*	2.0 ± 0.0*	2.0 ± 0.0*
	Polymorphonuclear Leukocytes		
1	0	0	0
3	0	0	0
6	2.5 ± 0.5*	1.5 ± 0.3*	1.2 ± 0.3*
24	4.0 ± 0.0*	2.0 ± 0.0*	2.0 ± 0.0*
	Red Blood Cells		
1	0	0	0
3	0	0	0
6	0	0	0
24	2.5 ± 0.2*	2.0 ± 0.0*	2.0 ± 0.0*
	Macrophages		
1	0	0	0
3	0	0	0
6	3.2 ± 0.3*	2.0 ± 0.0*	1.0 ± 0.0*
24	3.8 ± 0.2*	2.0 ± 0.0*	2.0 ± 0.0*

Table 7a: Histopathologic changes in the lung at various times after exposure to 13.9 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

	Type II Cell Hyperplasia		
1	0	0	0
3	0	0	0
6	0	0	0
24	0.8 ± 0.5	0.7 ± 0.4	0.3 ± 0.2
	Peribronchiolar Congestion		
1	0	0	0
3	0	0	0
6	0	0	0
24	1.7± 0.3*	1.5 ± 0.3*	1.2 ± 0.3*

Table 7b: Histopathologic changes in the lung at various times after exposure to 13.9 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

Exposure Concentration 18 ppm	Distribution	Severity	Intensity
Sacrifice Time (hr)	**Edema**		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
6	0	0	0
24	0.7 ± 0.5	0.7 ± 0.4	0.7 ± 0.4
	Fibrin		
1	0	0	0
2	0	0	0
3	1.2 ± 0.4*	1.3 ± 0.4*	0.7± 0.2*
4	3.7 ± 0.2*	2.0 ± 0.0*	1.0 ± 0.0*
6	4.0 ± 0.0*	2.0 ± 0.0*	1.3 ± 0.2*
24	4.0 ± 0.0*	2.2 ± 0.2*	2.0 ± 0.0*
	Polymorphonuclear Leukocytes		
1	0	0	0
2	0	0	0
3	0	0	0
4	1.0 ± 0.6	0.7 ± 0.4	0.3 ± 0.2
6	3.2 ± 0.3*	2.0 ± 0.0*	1.3 ± 0.2*
24	3.0 ± 0.0*	2.0 ± 0.0*	1.8 ± 0.2*

Table 8a: Histopathologic changes in the lung at various times after exposure to 18.6 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

Exposure Concentration 18 ppm	Distribution	Severity	Intensity
Sacrifice Time (hr)	**Red Blood Cells**		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
6	0	0	0
24	1.7± 0.3*	1.7± 0.3*	1.5 ± 0.3*
	Macrophages		
1	1.0 ± 0.4	0.7± 0.3	0.5 ± 0.2
2	0.2 ± 0.2	0.3 ± 0.3	0.2 ± 0.2
3	0.3 ± 0.2	0.7 ± 0.4	0.5 ± 0.3
4	2.7 ± 0.2*	2.0 ± 0.0*	1.2 ± 0.2*
6	2.8 ± 0.3*	2.0 ± 0.0*	1.2 ± 0.2*
24	4.0 ± 0.0*	2.3 ± 0.2*	2.0 ± 0.0*
	Type II Cell Hyperplasia		
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
6	0	0	0
24	2.2 ± 0.5*	1.5 ± 0.3*	1.2 ± 0.3*

Table 8b: Histopathologic changes in the lung at various times after exposure to 18.6 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

Exposure Concentration 23.3 ppm	Distribution	Severity	Intensity
Sacrifice Time (hr)	**Fibrin**		
1	0	0	0
4	3.0 ± 0.4*	2.0 ± 0.0*	2.0 ± 0.0*
24	0	0	0
	Polymorphonuclear Leukocytes		
1	0	0	0
4	2.3 ± 0.6*	1.7± 0.3*	1.2 ± 0.3*
24	0	0	0

Table 9: Histopathologic changes in the lung at various times after exposure to 23.3 ppm phosgene. (*): denotes values are significantly higher than corresponding values obtained from air-exposed control rats, $p<0.05$.

exposure. We presently do not have an explanation for this experimental outcome in this study component.

Biochemical Changes in Bronchoalveolar Lavage Fluids (BALF) after Phosgene Exposure: Bronchoalveolar lavage studies were performed with groups of rats that were exposed to two concentrations of phosgene, 9.3 ppm and 13.9 ppm. These two concentrations of phosgene were selected for these studies because of their uniquely different post-exposure latency periods. It should be noted here that no evidence was obtained in our studies that indicated that post-exposure exercise following exposure to air only, or the inhalation of 5% CO_2 during air exposures, results in increases in lavage fluid protein concentrations or lactate dehydrogenase (LDH) activities up to 24 hrs after exposure. Accordingly, all of the lavaged protein data and the LDH data from the various experiments involving bronchoalveolar lavage of air-exposed animals were pooled for statistical comparisons with data obtained from phosgene-exposed groups of rats.

BALF Protein Changes

Total protein concentrations in the lavage fluids of rats that were exposed to air or 9.3 ppm phosgene for 10 min and then sacrificed at various times after exposure are summarized in Figure 10. No significant increases in lavagable protein concentration were found as of 1 hr after exposure to 9.3 ppm phosgene compared to the lavagable protein concentration from animals exposed to air only. As of 3 hrs after phosgene exposure, however, BALF protein concentrations had increased ~3-fold. Similar elevations in BALF protein continued to be present as of 6 hrs after exposure. Thereafter, BALF protein continued to progressively increase so that by 10 hrs after exposure to the 9.3 ppm concentration of phosgene protein concentrations had increased ~15-fold and as of 24 hrs after exposure BALF protein further increased ~30-fold. HPLC analyses of the lavage fluids confirmed that the protein increases were indeed associated with increases in lavageable albumin and transferrin.

Changes in BALF protein concentrations following exposure to 13.9 ppm phosgene are summarized and compared to BALF protein changes following exposure to the 9.3 ppm concentration of phosgene in Figure 11. Unlike with the 9.3 ppm concentration of phosgene, BALF protein was significantly elevated as of 1 hr after exposure to the higher phosgene concentration. As of 3 hr after exposure to 13.9 ppm phosgene, protein concentration in the lavage fluid of rats further increased to values that were ~5-fold greater than those obtained from air control rats. Similar elevations in BALF protein remained present as of 6 hr after inhalation of the 13.9 ppm concentration of

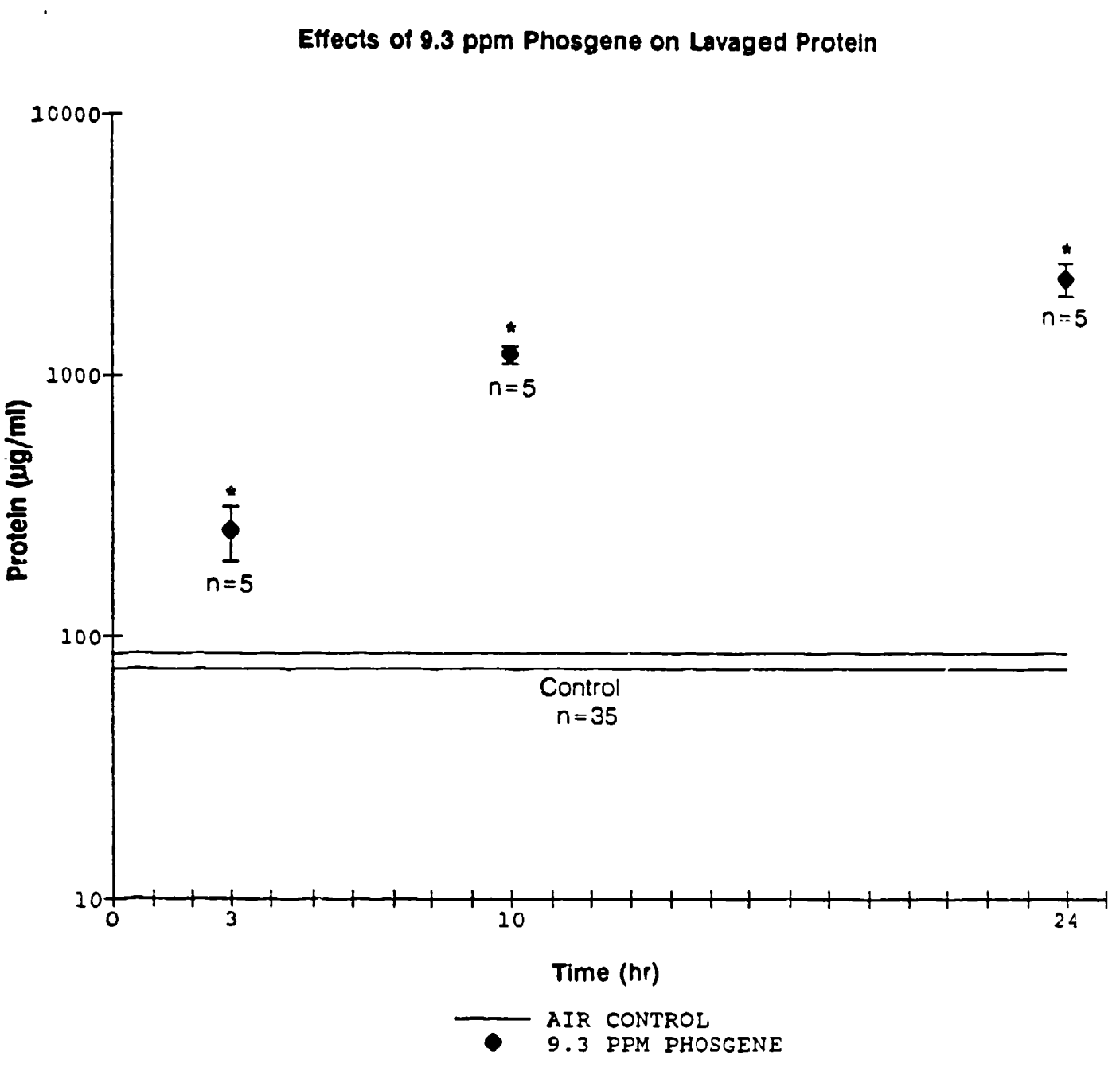

Figure 10. Effects of 10 minute exposure to 9.3 ppm phosgene on protein concentrations in lavage fluids. Significant increases (*) in lavageable protein were found as early as 3 hours after exposure to this concentration of phosgene ($p \leq 0.05$).

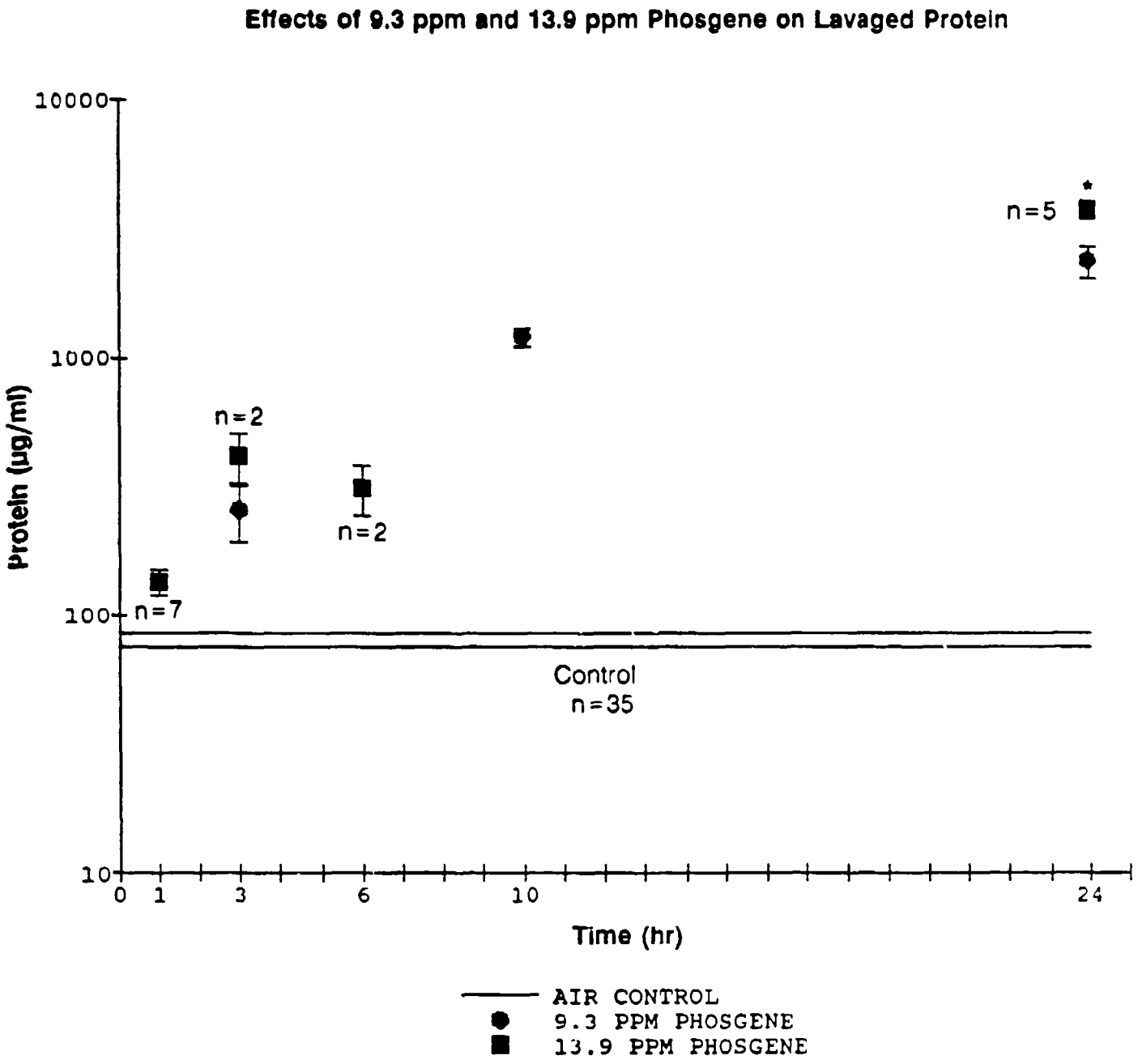

Figure 11. Effects of exposure to either 9.3 ppm or 13.9 ppm phosgene for 10 minutes on lavageable protein concentrations. Protein concentrations as of 24 hours after exposure (*) were significantly different ($p \leq 0.05$).

phosgene. Thereafter, the concentration of protein in BALF progressively increased so that by 24 hrs after exposure the lavaged protein concentration was ~46-fold greater than in control lavage fluids. Relative to the 9.3 ppm phosgene concentration, the 13.9 ppm concentration of phosgene brought about an ~1.6-fold greater protein response as of the last 24 hr post-exposure sacrifice time. HPLC analyses of the lavage fluids confirmed that the protein increases following the 13.9 ppm phosgene exposures were indeed associated with corresponding increases in lavageable albumin and transferrin.

BALF Lactate Dehydrogenase Activity Changes

Lactate dehydrogenase (LDH) concentration measured in the lavage fluid of rats exposed to air, 9.3 ppm, or 13.9 ppm phosgene and sacrificed at various times after exposure are illustrated in Figures 12 and 13. Following exposure to both concentrations of phosgene, significant elevations in LDH activity was observed as of 1 hr after the exposures with values obtained for both concentrations being similar. Maximal increases in LDH activity occurred at the 24 hr time points in these studies. As of this last sacrifice time, the 13.9 ppm exposure resulted in significantly higher LDH activities in BALF compared to BALF LDH activities following the 9.3 ppm exposures.

BALF Cellular Changes after Phosgene Exposure: Cell types and numbers harvested by bronchoalveolar lavage were also determined following the 9.3 ppm and 13.9 ppm phosgene exposures. As with the lavage fluid biochemical constituents, it is noted here that no evidence was obtained in our studies that indicated: 1) that the exposure of rats to air only causes changes in the numeric composition of the lung's free cell population relative to cage-control animals, 2) that the exposure rats to air + CO_2 causes changes in the numeric composition of the lung's free cell population, or 3) that post-exposure exercise causes changes in the numeric composition of the lung's free cell population for up to 24 hrs after exposure. Accordingly, all of the lavaged cell data data obtained from the various experiments involving bronchoalveolar lavage of air-exposed, air + CO_2-exposed, and cage control animals were pooled (Table 10) for statistical comparisons with data obtained from phosgene-exposed groups of rats.

As indicated in Table 10, virtually all of the cells lavaged from the lungs of healthy control animals were alveolar macrophages. No significant changes in the numbers of lavaged alveolar macrophages (AM), polymorphonuclear leukocytes (PMN), lymphocytes (Lymph), monocytes (Mono), or eosinophils (Eos) occurred as of 1 hr after exposure to the 9.3 ppm concentration of phosgene, Table 11. As of 3 hrs after phosgene exposure, however, the total numbers of cells harvested was significantly reduced from control

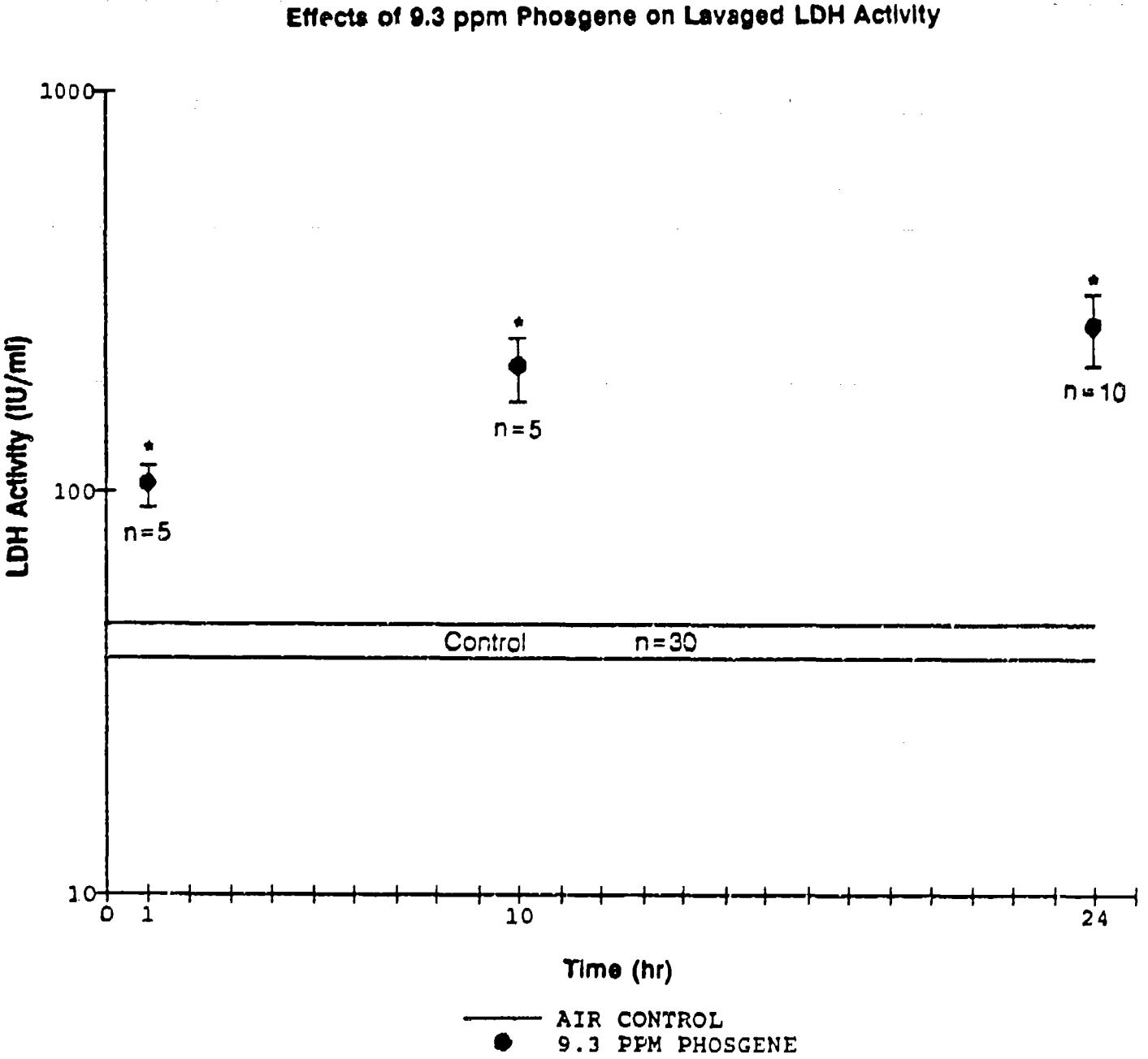

Figure 12. Effects of exposure to 9.3 ppm phosgene for 10 minutes on lavageable LDH activity. Significant increases (*) in LDH activity relative to air control values occurred as early as 1 hour after exposure to this concentration of phosgene ($p \leq 0.05$).

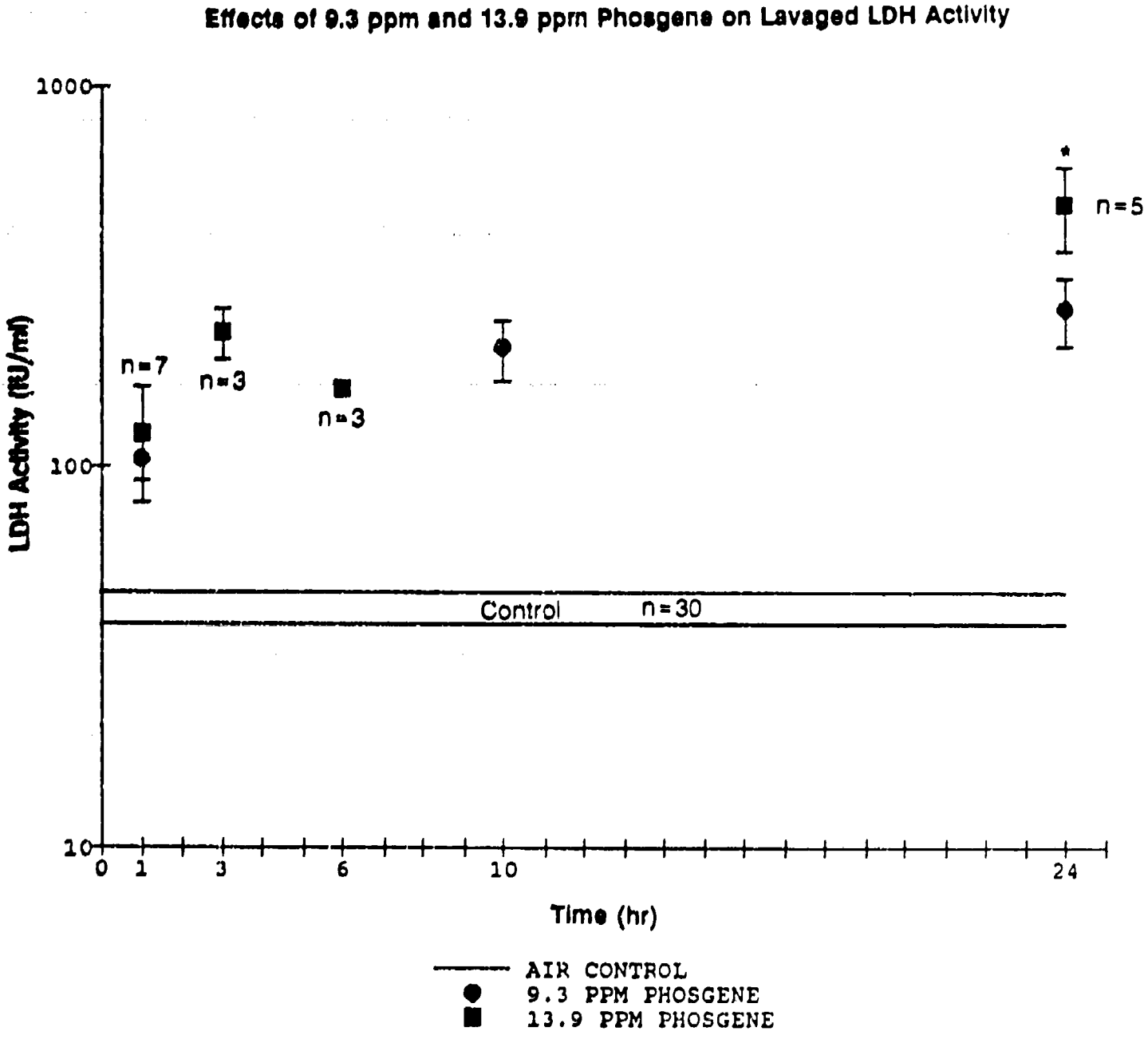

Figure 13. Effects of exposure to either 9.3 ppm or 13.9 ppm phosgene for 10 minutes on lavageable LDH activity. LDH activities as of 1 hour after exposure to the two different concentrations of phosgene were not significantly different, however LDH activities as of 24 hours after exposure (*) were significantly different ($p \leq 0.05$).

Table 10 Numbers ($x10^{-7}$) of Different Cell Types Lavaged from the Lungs of Air-Exposed and Cage Control Rats

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.490±0.036	1.470±0.036	0.007±0.001	0.022±0.006	0.006±0.001	0	27

Table 11 Numbers ($x10^{-7}$) of Different Cell Types Lavaged at Various Times after Exposure to 9.3 ppm Phosgene

			1 hr			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.498±0.036	1.483±0.037	0.004±0.002	0.008±0.002	0.003±0.002	0	5
			3 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.248±0.047**	1.221±0.044**	0.0149±0.004	0.002±0.001	0.009±0.003	0	5
			10 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.156±0.066**	1.111±0.067**	0.018±0.004*	0.019±0.007	0.006±0.001	0.001±0.001	5
			24 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.537±0.134	1.052±0.085**	0.238±0.039*	0.116±0.027*	0.130±0.025*	0.001±0.001	10

Total Cells: all lavaged cells; **AM**: alveolar macrophages; **PMN**: polymorphonuclear leukocytes; **Lymph**: lymphocytes; **Mono**: monocytes/small mononuclear cells; **Eos**: eosinophils; **n**: numbers of animals studied. *: significantly higher than control values ($p<0.05$). **: significantly lower than control values ($p<0.05$).

levels, with such reductions being due to a decrease in the numbers of lavaged AM. Further decreases in AM numbers appeared to progressively continue thereafter so that AM numbers harvested as of the last 24 hr time point were ~70% of control values. PMN numbers, on the other hand, were significantly elevated as of 10 hrs after exposure to 9.3 ppm phosgene, and, as of 24 hrs after exposure, these inflammatory cells accounted for ~15% of the total lung free cell populations. Other notable observations made in the cell studies with this phosgene concentration were that the AM took on a highly vacuolated appearance as of 10 hrs after phosgene exposure, and that erythrocytes were abundantly present as of this time point. Additionally, the AM showed a propensity to clump as of the last 24 hr time point, the cytoplasm and nuclei of many AM were vacuiated, and clumps of platelets were present in some of the lavaged samples.

Changes in the lung free cell population following exposure to the 13.9 ppm concentration of phosgene are summarized in Table 12. This concentration of phosgene caused significant reductions in the total size of the lavaged populations as of 1 hr after exposure due to decreases in the numbers of lavaged AM; at this earliest post-exposure time, some of the AM were "foamy" in appearance. However, total cell numbers and AM numbers harvested as of the 3 and 6 hr post-exposure time points were not significantly different from control values. In that no detectable increases in monocytes were noted at these times to suggest the recruitment of additional mononuclear phagocytes into the alveolar space compartment, the earlier decrease in AM numbers observed at the 1 hr time point may have been due in a decrease in their lavageability, e.g., a change in their *in situ* adherence characteristics. Many of the AM were noted to be highly vaculoated as of the 6 hr time point, and "smudge cells" of unknown type were prevalent on the slide preparations. As of the last 24 hr time point, total cell numbers were significantly elevated, while AM numbers continued to be decreased. The increase in the total cell numbers were attributable to significant increases in PMN, Lymph, and Mono, which accounted for ~31%, ~10% and ~15%, respectively, of all the lavaged cells. Also, many of the slide specimens at the last sacrifice time point contained large numbers of erythrocytes and clumps of AM.

Collectively, these results suggest that the lung's AM are a target of phosgene. Whether the vacuolization and the evidence for a change in the adherence properties of AM reflect a direct cellular toxicity or, instead, a secondary effect resulting from the stimulated release of other mediators that in turn affect the AM requires further study.

Table 12 Numbers ($x10^{-7}$) of Different Cell Types Lavaged at Various Times after Exposure to 13.9 ppm Phosgene

			1 hr			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
0.882±0.095**	0.872±0.093**	0.003±0.002	0.005±0.002	0.002±0.001	0.001±0.001	6
			3 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.373±0.124	1.348±0.123	0.009±0.003	0.007±0.001	0.008±0.003	0.002±0.002	3
			6 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
1.060±0.197	0.960±0.212	0.074±0.019	0.019±0.003	0.006±0.001	0	3
			24 hrs			
Total Cells	**AM**	**PMN**	**Lymph**	**Mono**	**Eos**	**(n)**
2.354±0.133*	1.034±0.110**	0.737±0.101*	0.230±0.027*	0.359±0.043*	0.004±0.003	5

Total Cells: all lavaged cells; **AM**: alveolar macrophages; **PMN**: polymorphonuclear leukocytes; **Lymph**: lymphocytes; **Mono**: monocytes/small mononuclear cells; **Eos**: eosinophils; **n**: numbers of animals studied. *: significantly higher than control values ($p<0.005$). **: significantly lower than control values ($p<0.005$).

Minute Ventilation and Phosgene Toxicity

Ventilatory Responses to Phosgene in the Presence and Absence of CO_2: Normal ventilatory function profiles during air breathing and air + 5% CO_2 inhalation are summarized in Figures 14-16. Figure 12 represents minute ventilation (V_E) measurements on a group of animals before, during and after an exposure to air or to air + 5% CO_2. Upon the inhalation of 5% CO_2, V_E increases ~70% due exclusively to increases in tidal volume (V_t), Figure 15; breathing frequency (f) is normally not changed due to 5% CO_2 inhalation, Figure 16.

Ventilatory patterns before, during, and after exposure to 9.3 ppm phosgene or 9.3 ppm phosgene + 5% CO_2 are shown in Figures 17-19. Upon the inhalation of 9.3 ppm phosgene, V_E became rapidly reduced, approximately 38%. The reduction in V_E was the result of an immediate reduction in V_t of ~60%, and an increase in f of approximately 30%. Overall V_E remained depressed over the 10 min exposure to 9.3 ppm phosgene, while V_t slowly increased and f slowly decreased over the exposure duration. After exposure, f values became similar to control levels, while V_t values remained depressed. This resulted in an reduction in V_E of approximately 31% 10 min after the end of the 9.3 ppm phosgene exposure.

WIth the addition of 5% CO_2 during the 9.3 ppm phosgene exposure, V_E was immediately reduced to values similar to values with 9.3 ppm phosgene only, but then rapidly increased to a steady state that was still somewhat depressed from control values (~18%). The increase in V_E due to 5% CO_2 inhalation during 9.3 ppm phosgene exposure was approximately 30% higher compared to inhalation of 9.3 ppm phosgene alone, or approximately half of the normal ventilatory response expected due to 5% CO_2 inhalation. V_E elevations during the phosgene + 5% CO_2 inhalation exposure were primarly due to increases in V_t and slight increases in f. A trend of increasing values for V_E during 9.3 ppm phosgene + 5% CO_2 was apparant toward the end of the exposure. This increase was exclusively due to increases in V_t. After the exposure to 9.3 ppm phosgene + 5% CO_2, values of V_E remained somewhat depressed compared to pre-exposure values, but they were not as diminished as V_E values after exposure to 9.3 ppm phosgene alone.

Ventilatory patterns before, during and after exposure to 13.9 ppm phosgene or 13.9 ppm phosgene + 5% CO_2 are shown in Figures 20-22. Like V_E values measured with animals exposed to 9.3 ppm phosgene, V_E became rapidly reduced by ~37% upon the inhalation of the 13.9 ppm phosgene concentration. The reduction in V_E was the result of an immediate reduction in V_t of ~53%, and an increase in f of ~35%. Overall V_E remained

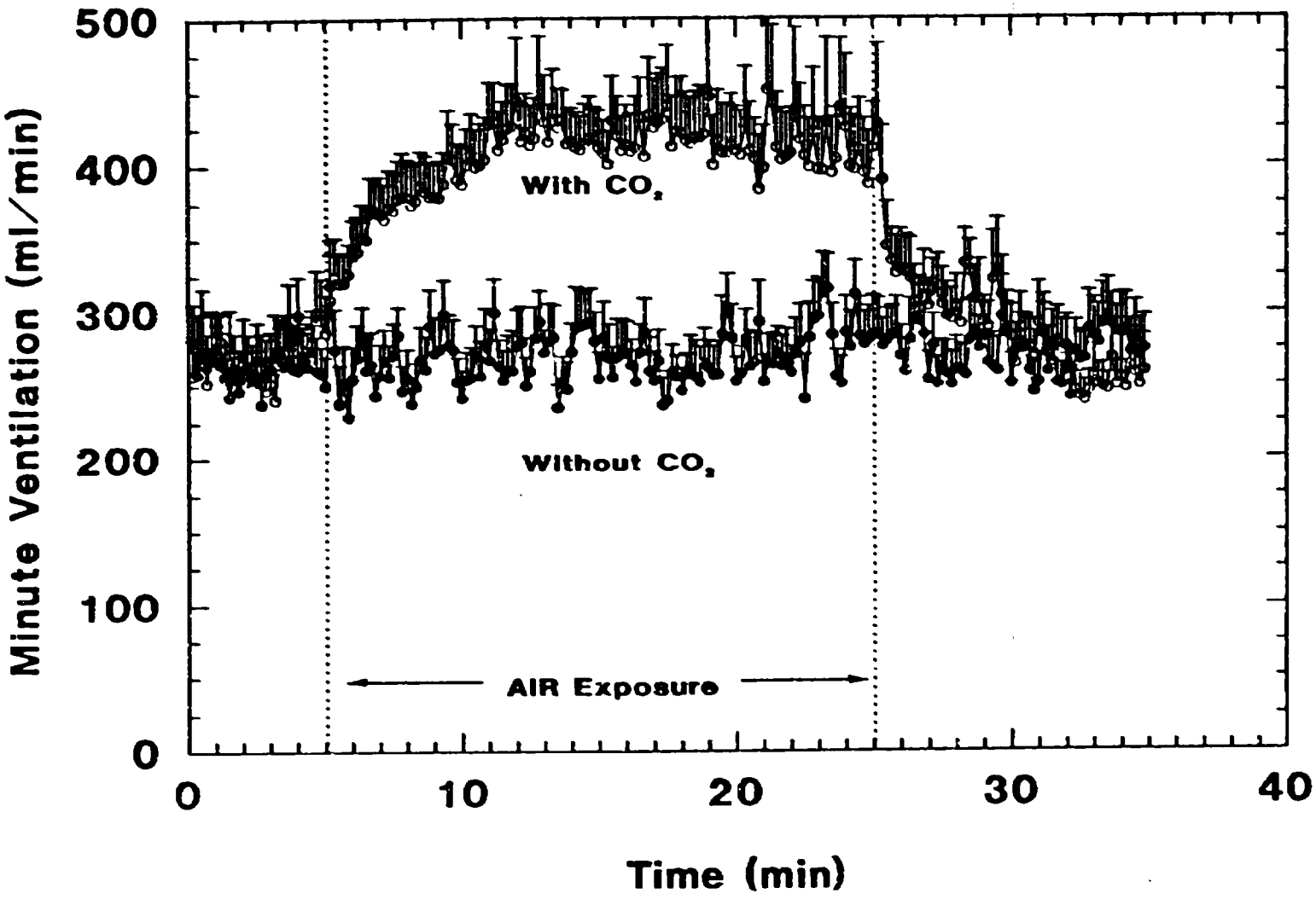

Figure 14 Minute ventilation (V_E) measured on rats before, during and after exposure to air or air + 5% CO_2. Each point represents the Mean and S.E.M. of average minute ventilation values of a 10 second period of N=6 rats.

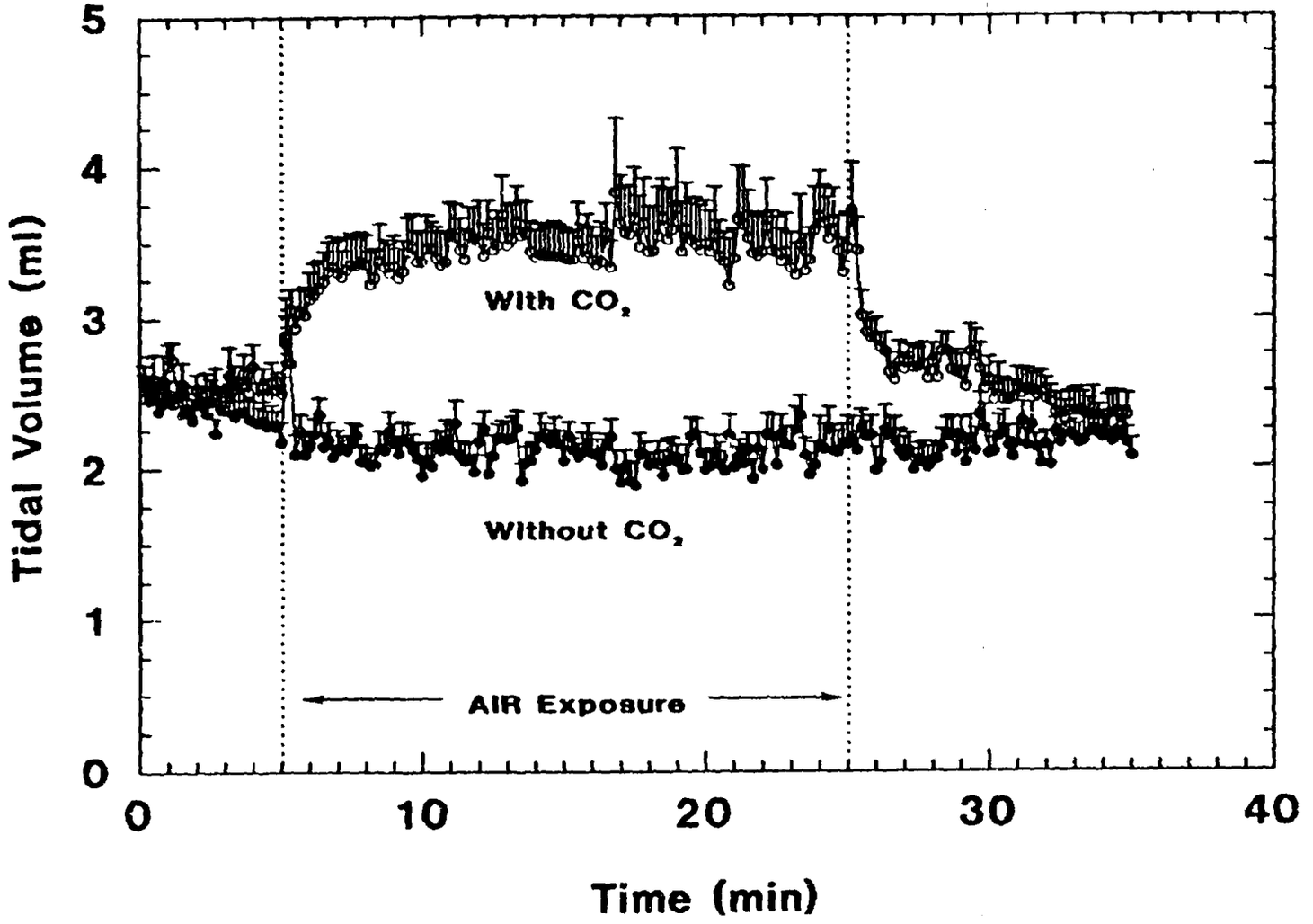

Figure 15 Tidal Volume (V_T) measured on rats before, during and after exposure to air or air + 5% CO_2. Each point represents the Mean and S.E.M. of average tidal volume values of a 10 second period of N=6 rats.

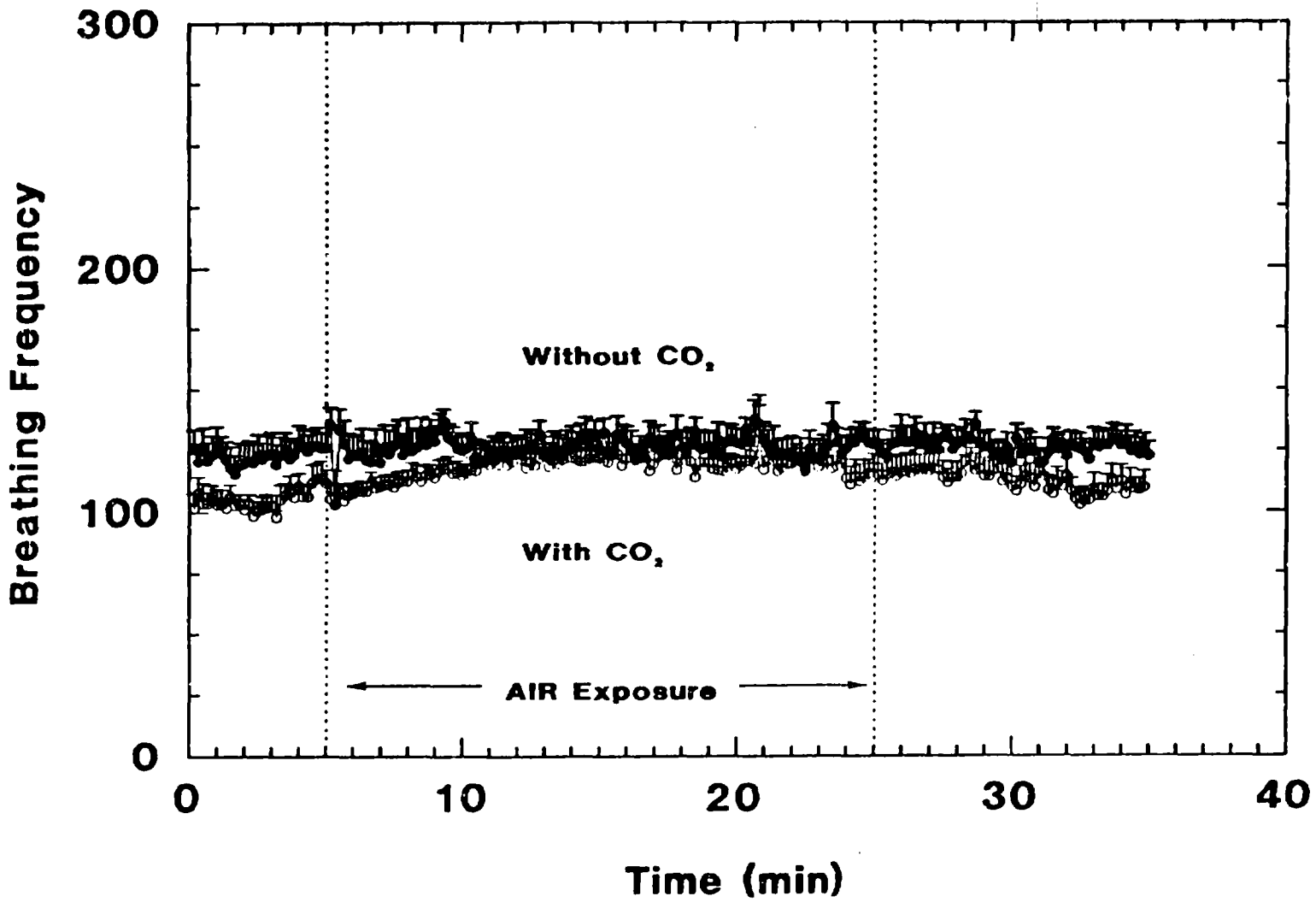

Figure 16 Breathing frequency (f) measured on rats before, during and after exposure to air or air + 5% CO_2. Each point represents the Mean and S.E.M. of average breathing frequency values of a 10 second period of N=6 rats.

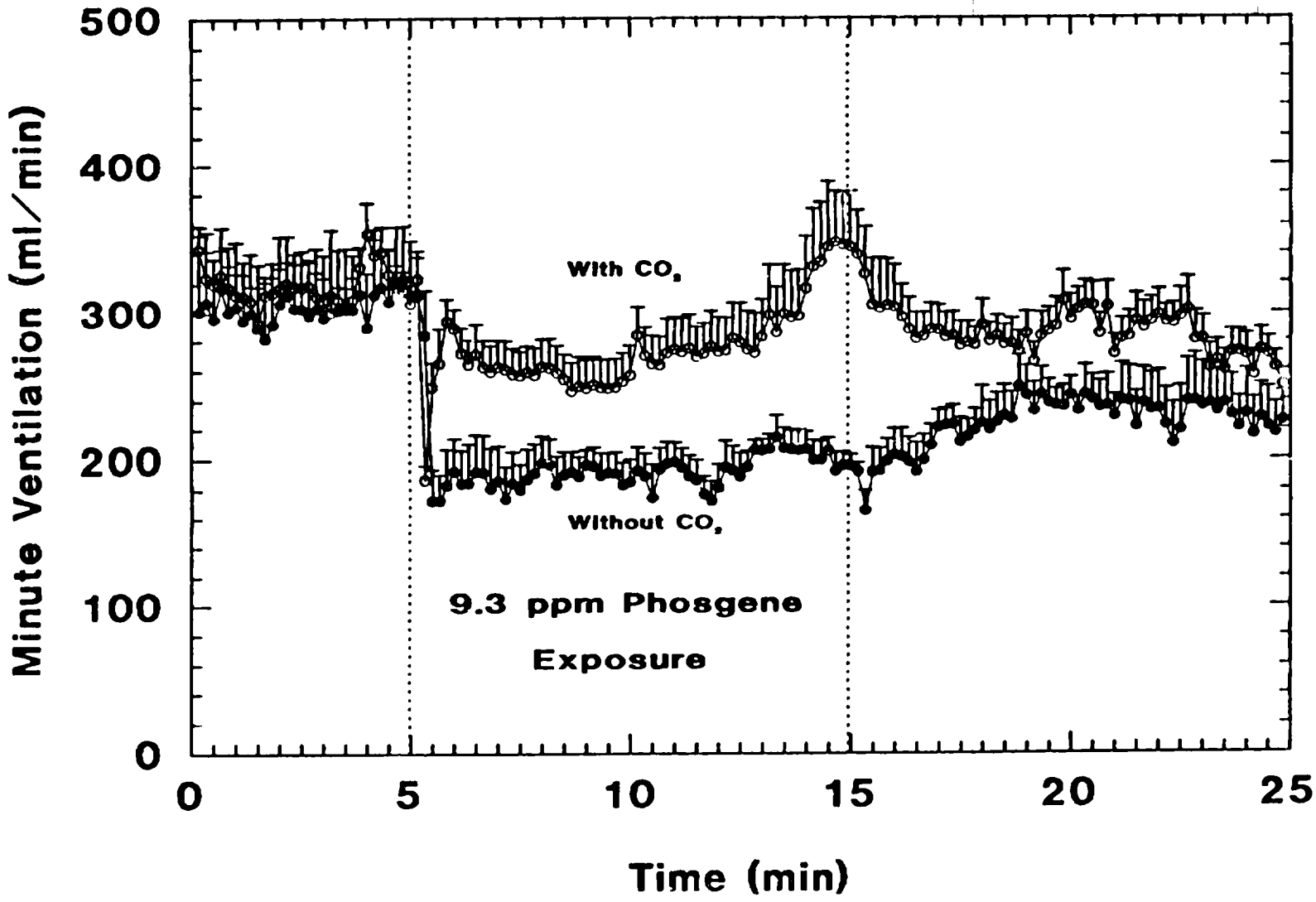

Figure 17 Minute ventilation (V_E) measured on rats before, during and after exposure to 9.3 ppm Phosgene or 9.3 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average minute ventilation values of a 10 second period of N=6-8 rats.

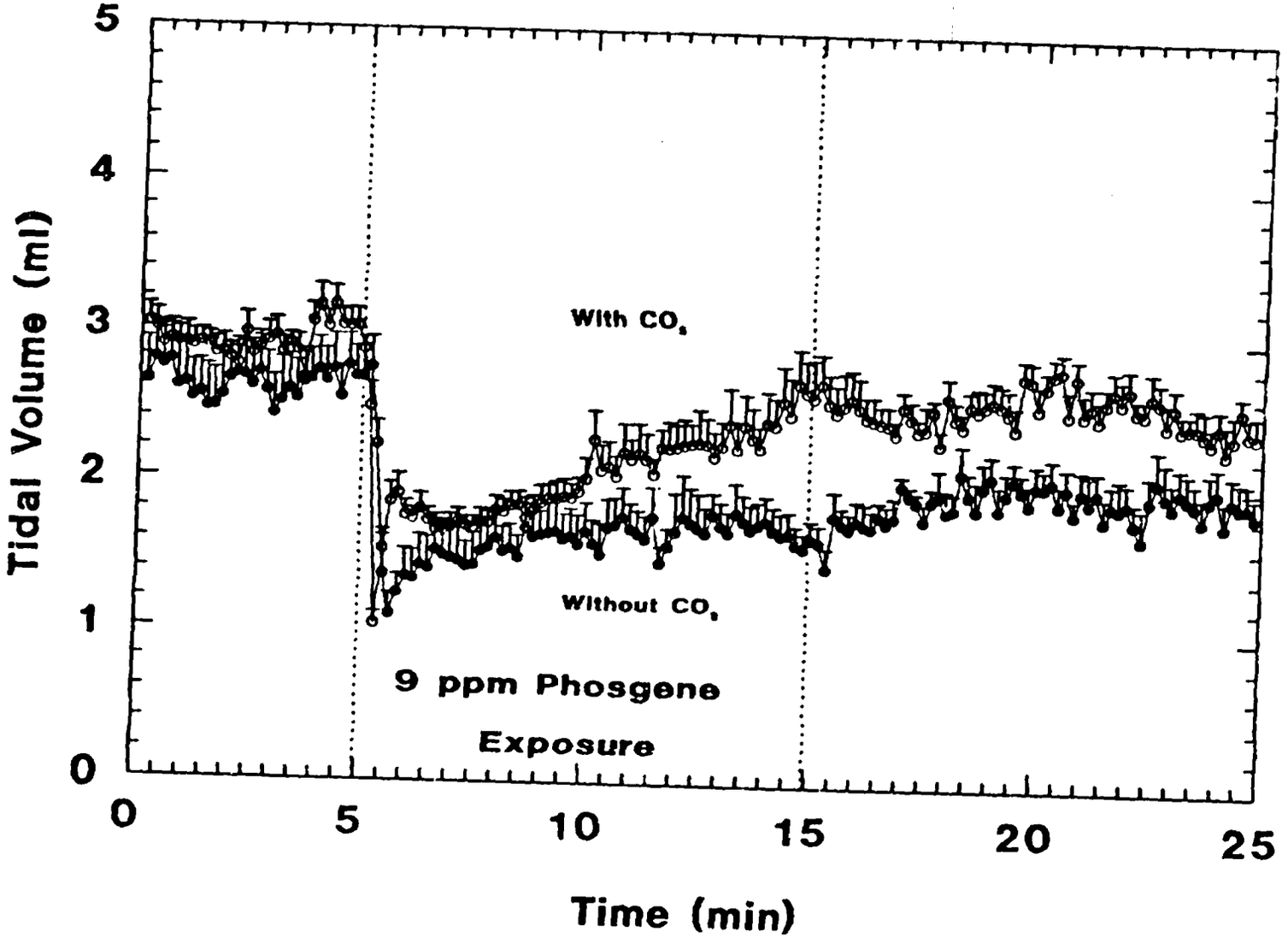

Figure 18 Tidal Volume (V_T) measured on rats before, during and after exposure to 9.3 ppm Phosgene or 9.3 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average tidal volume values of a 10 second period of N=6-8 rats.

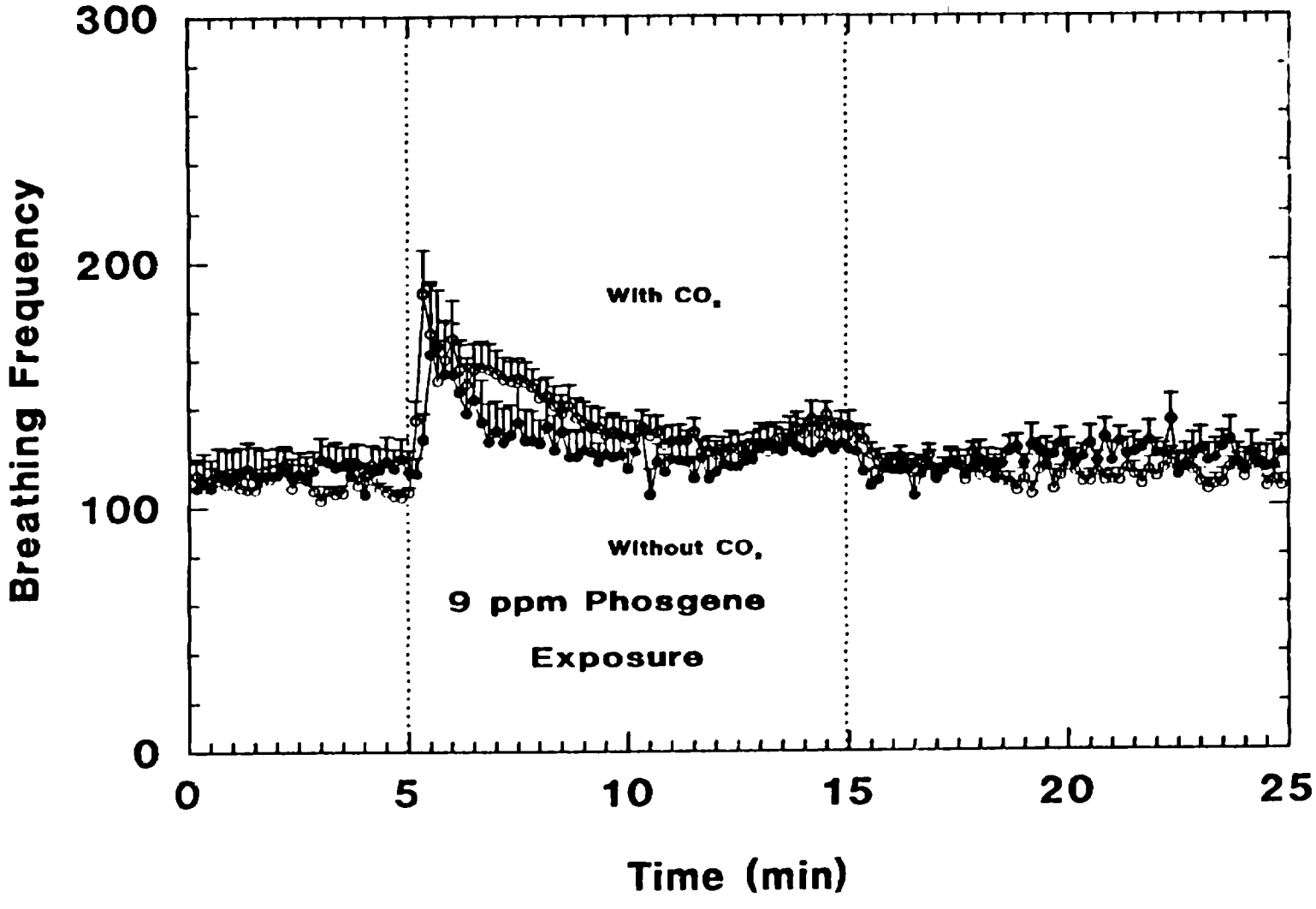

Figure 19 Breathing frequency (f) measured on rats before, during and after exposure to 9.3 ppm Phosgene or 9.3 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average breathing frequency values of a 10 second period of N=6-8 rats.

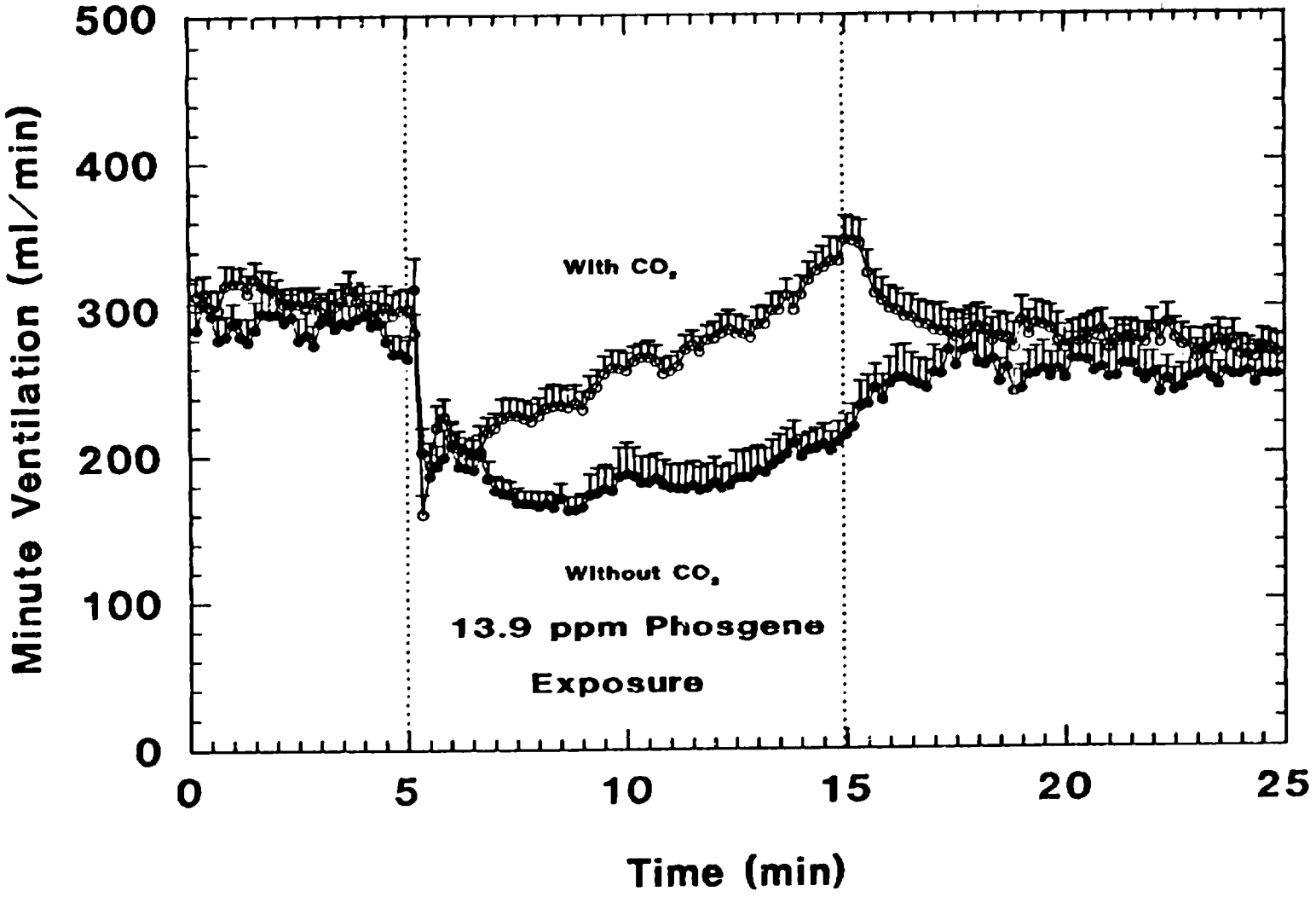

Figure 20 Minute ventilation (V_E) measured on rats before, during and after exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average minute ventilation values of a 10 second period of N=6 rats.

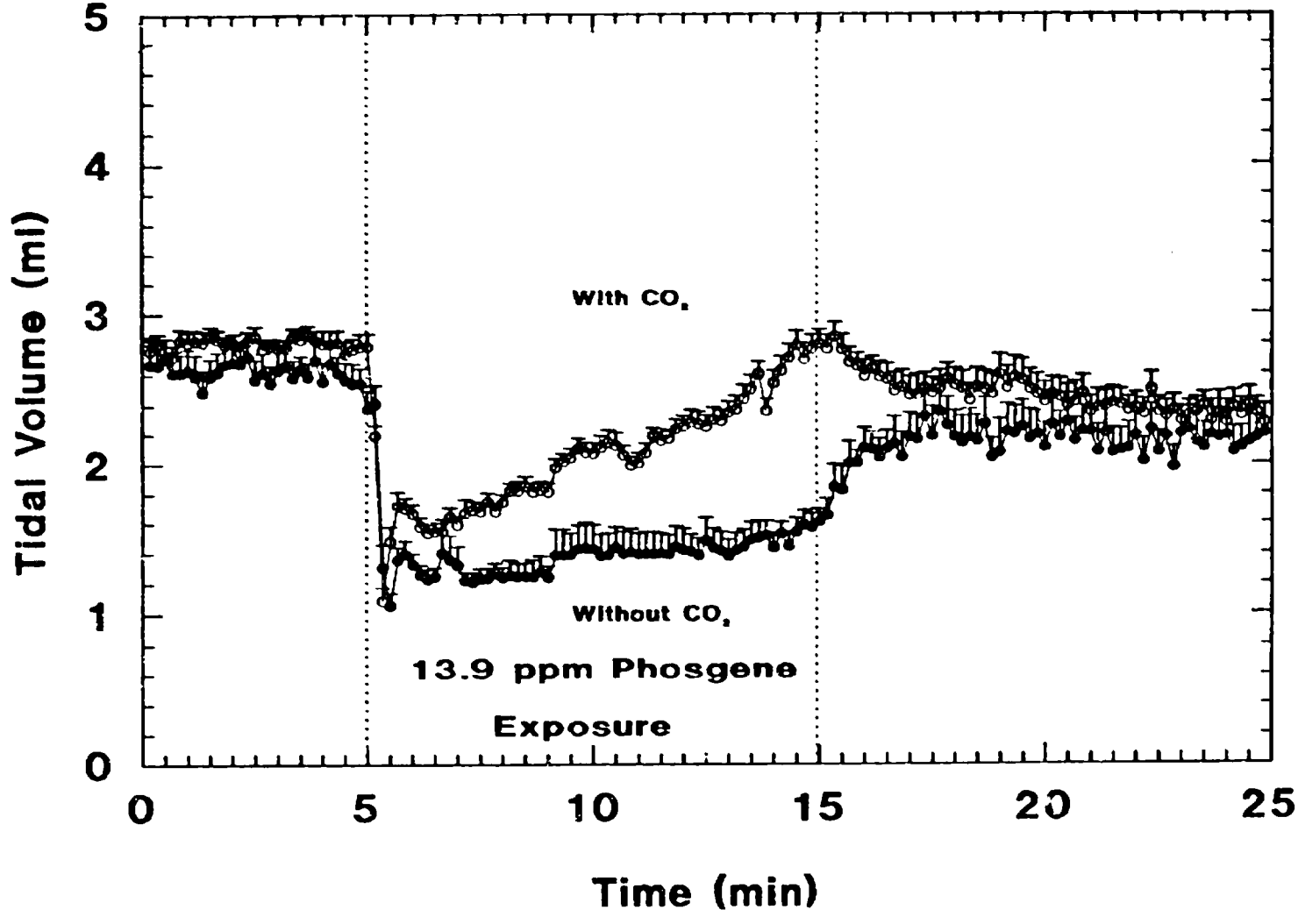

Figure 21 Tidal Volume (V_T) measured on rats before, during and after exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average tidal volume values of a 10 second period of N=6 rats.

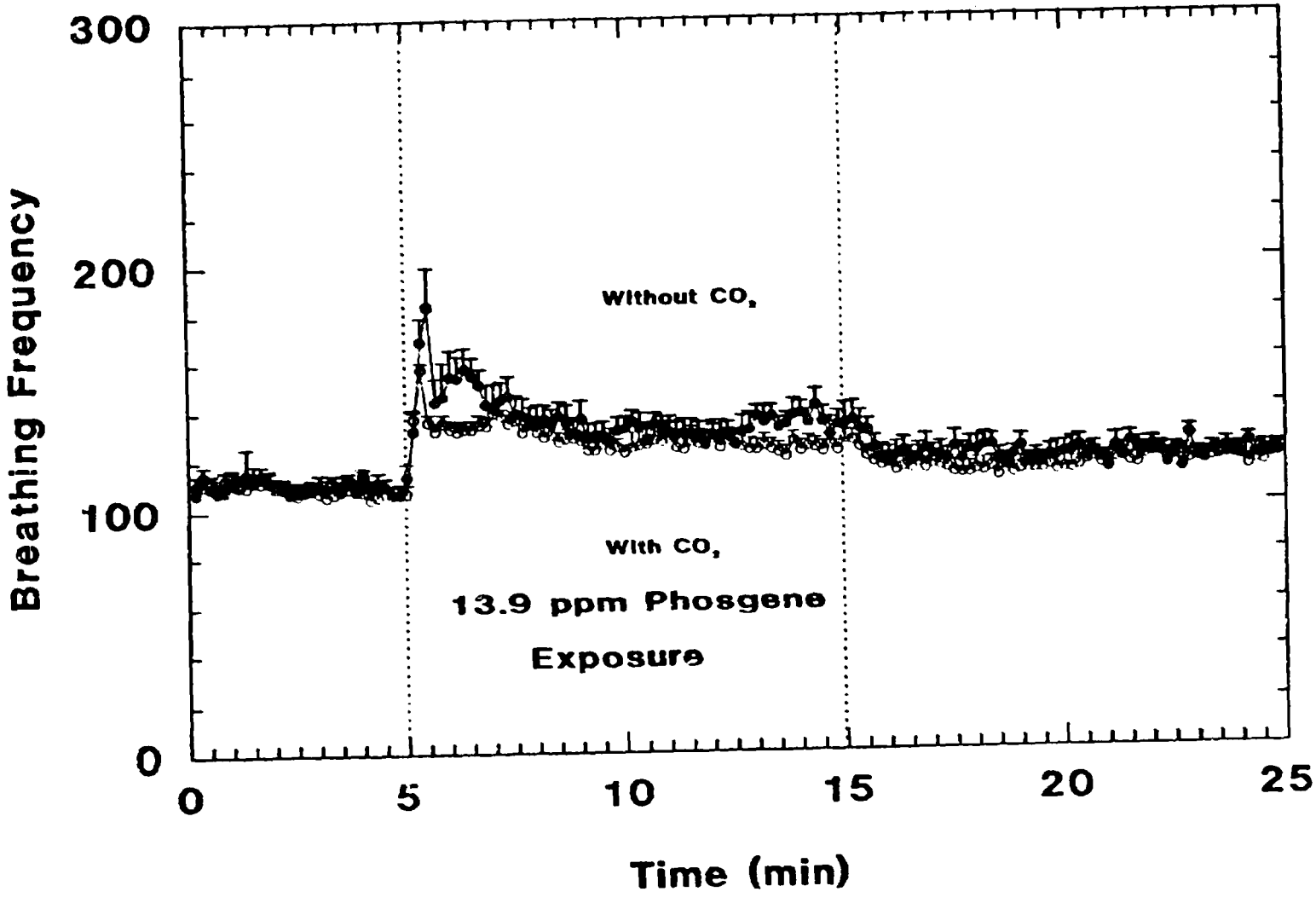

Figure 22 Breathing frequency (f) measured on rats before, during and after exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of average breathing frequency values of a 10 second period of N=6 rats.

depressed over the 10 min exposure to 13.9 ppm phosgene, while V_t slowly increased and f slowly decreased over the exposure duration. After exposure, f values were closely similar to control levels, while V_t values remained depressed. This resulted in a reduction in V_E of ~16% 10 min after the end of the phosgene exposure. With the addition of 5% CO_2 during the 13.9 ppm phosgene exposure, V_E became immediately reduced to values similar to values obtained with 13.9 ppm phosgene only. Thereafter, however, V_E slowly increased over the duration of the exposure, resulting in a 10% higher V_E at the end of the exposure compared to pre-exposure values. The V_E elevations that did occur during 5% CO_2 inhalation were due to increases in V_t. A trend of increasing values for V_E during 13.9 ppm phosgene + 5% CO_2 was apparent toward the end of the exposure exclusively related to increases in V_t. After the exposure to 13.9 ppm phosgene + 5% CO_2, values of V_E return to pre-exposure values.

Lung Gravimetric Changes Due to Phosgene Inhalation at Increased V_E: LWW and RCLDW measurements at various times after exposure to 9.3 ppm phosgene or 9.3 ppm phosgene + 5% CO_2 are summarized in Figures 23 and 24. As can be seen from these figures, the latency period during which no significant increases in LWW or RCLDW occur is reduced to 3-4 hrs post exposure when animals inhale phosgene during periods of increased V_E compared to the 6-7 hr latency period of animals exposed to 9.3 ppm phosgene at normal ventilatory rates. Also the magnitude of injury, measured by significantly greater increases in LWW and RCLDW at selected time points after exposure, is potentiated by the increased rates of ventilation displayed by animals exposed to 9.3 ppm phosgene + 5% CO_2, compared to animals exposed to phosgene only.

Increased V_E during inhalation of higher concentrations of phosgene elicits the same gravimetric response. Figures 25 and 26 demonstrate the LWW and RCLDW response of groups of animals exposed to 13.9 ppm phosgene with or without concurrent inhalation of 5% CO_2. The post-exposure latency period until LWW and RCLDW increases are observed is reduced several hours for animals exposed to 13.9 ppm phosgene + 5% CO_2, compared to animals exposed to this concentration of phosgene without CO_2. Also, LWW and RCLDW values were significantly greater in animals exposed to 13.9 ppm phosgene + 5% CO_2 compared to values measured with animals exposed to phosgene alone.

Increased V_E During Phosgene Exposure and BALF Biochemical Changes: The effects of increasing minute ventilation (V_E) during exposure to 13.9 ppm phosgene relative to BALF post-exposure protein concentrations obtained from rats that were

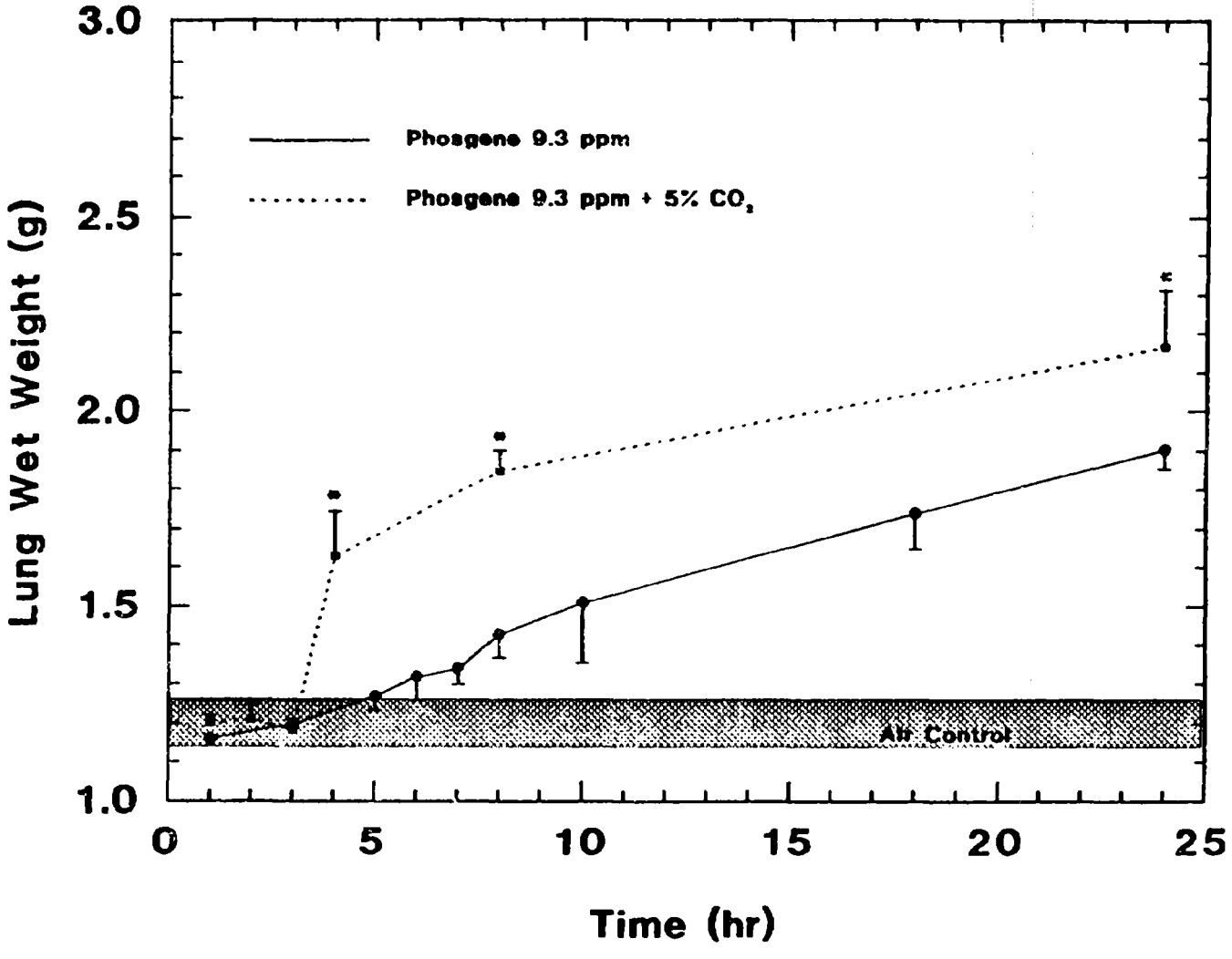

Figure 23 Lung Wet Weight (LWW) at various times after exposure to 9.3 ppm Phosgene or 9.3 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of N=5-8 rats. (*) indicates a significant increase in LWW compared to LWW values of Phosgene alone exposed animals, ($p \leq 0.05$). Error bars above the cross-hatch region indicate a significant elevation of LWW values compared to values from animals exposed to air only, ($p \leq 0.05$).

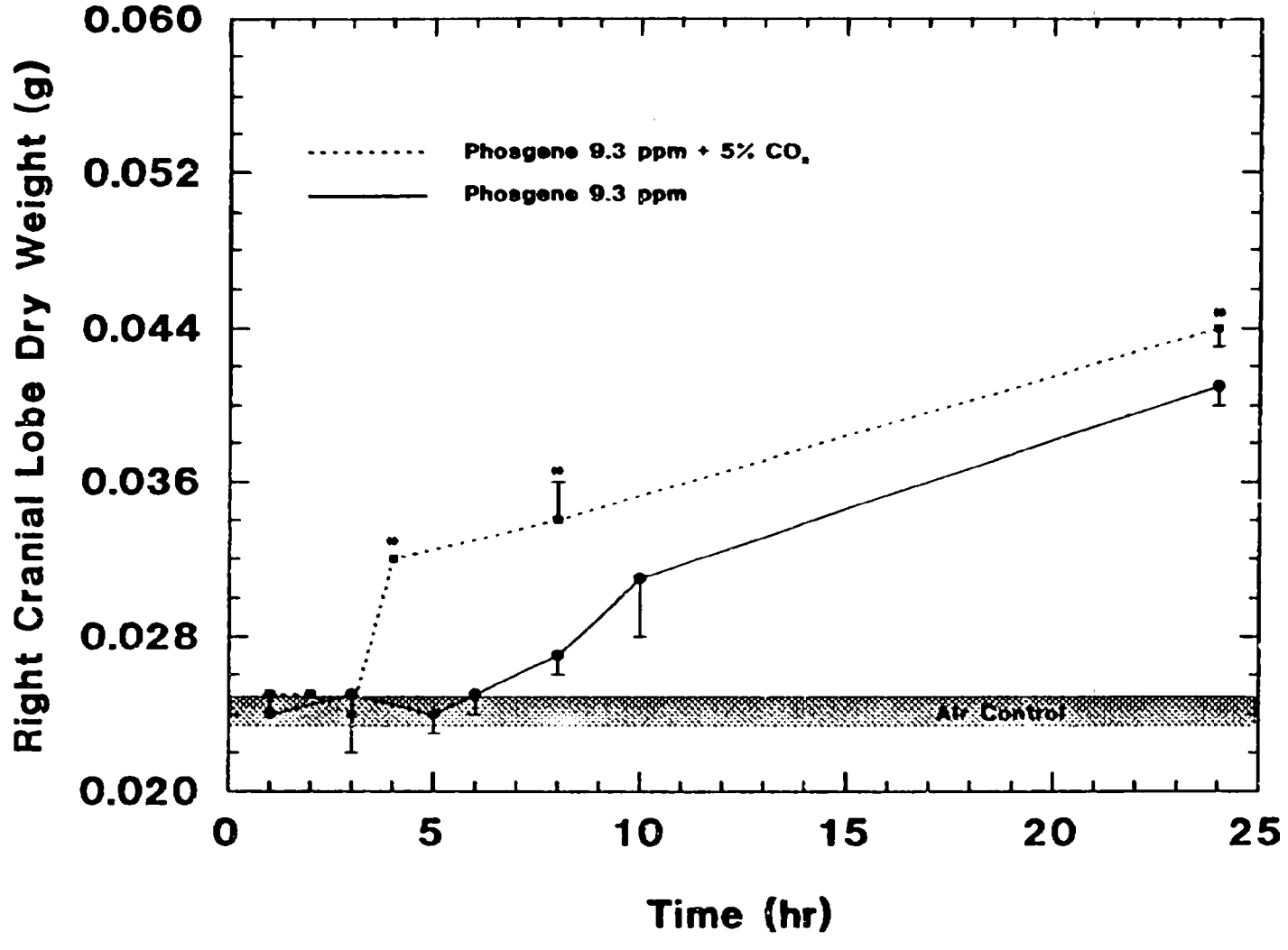

Figure 24 Right Cranial Lobe Dry Weight (RCLDW) at various times after exposure to 9.3 ppm Phosgene or 9.3 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of N=5-8 rats. (*) indicates a significant increase in RCLDW compared to RCLDW values of Phosgene alone exposed animals, ($p \leq 0.05$). Error bars above the cross-hatch region indicate a significant elevation of RCLDW values compared to values from animals exposed to air only, ($p \leq 0.05$).

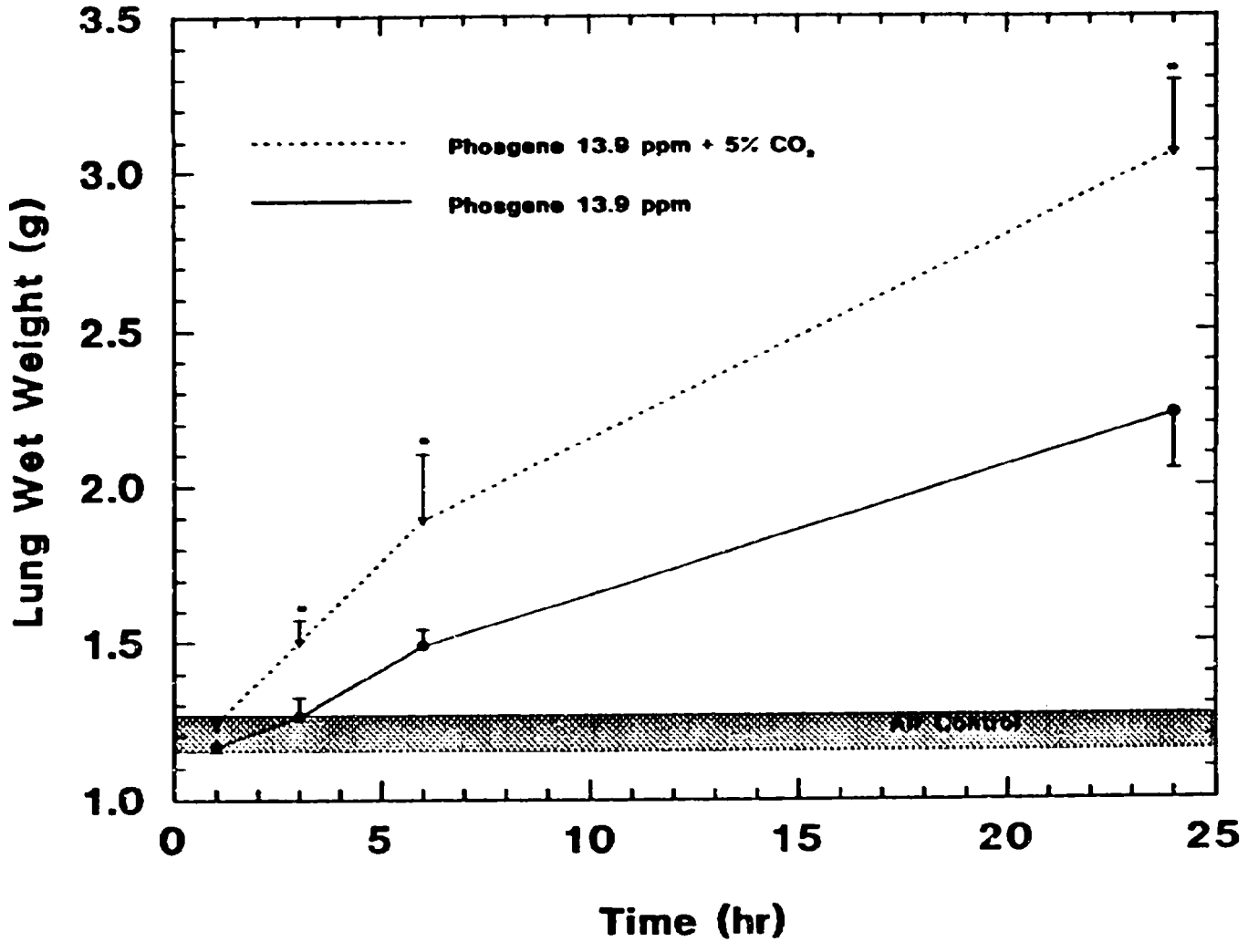

Figure 25 Lung Wet Weight (LWW) at various times after exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of N=5-6 rats. (*) indicates a significant increase in LWW compared to LWW values of Phosgene alone exposed animals, ($p \leq 0.05$). Error bars above the cross-hatch region indicate a significant elevation of LWW values compared to values from animals exposed to air only, ($p \leq 0.05$).

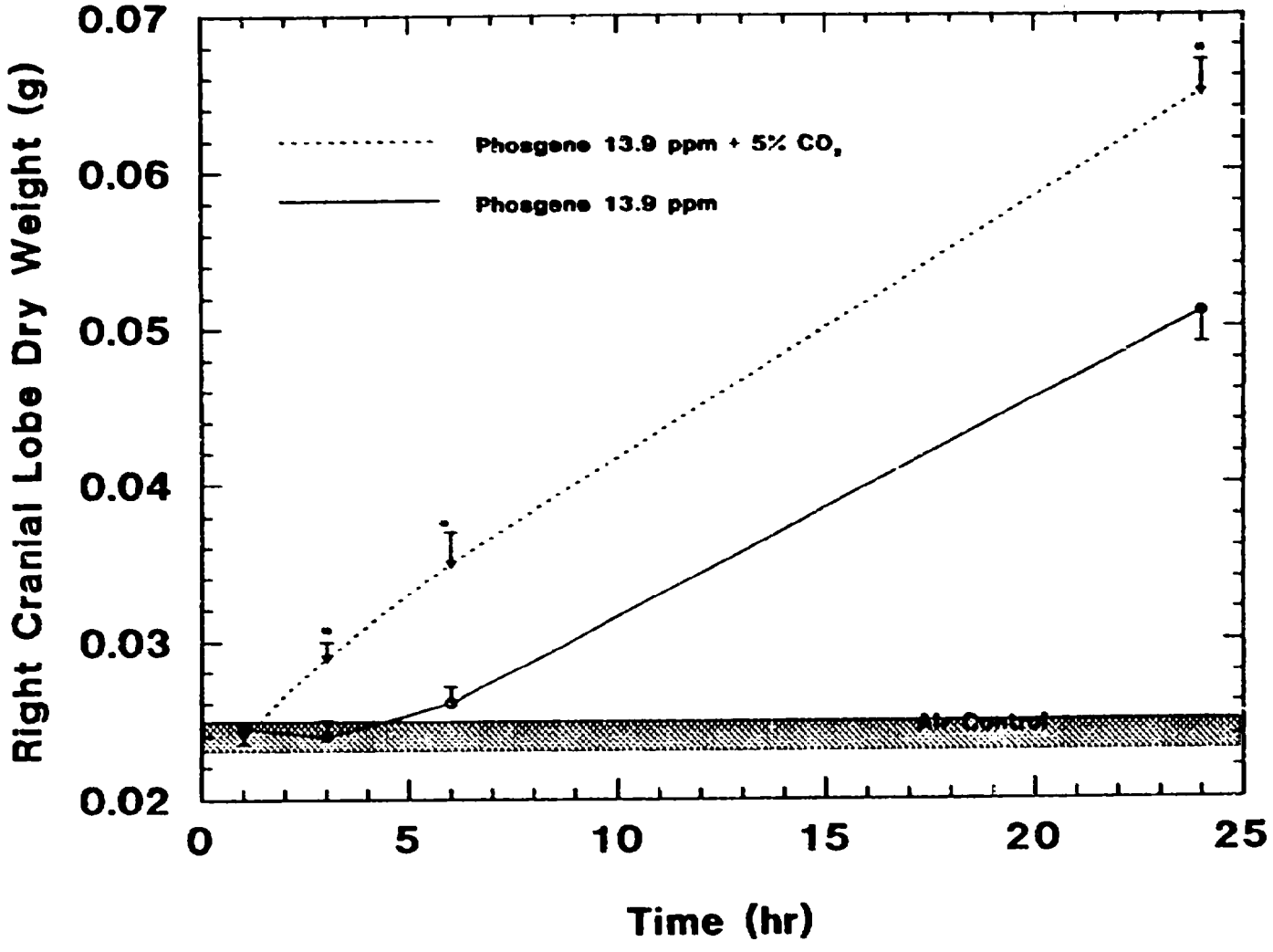

Figure 26 Right Cranial Lobe Dry Weight (RCLDW) at various times after exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2. Each point represents the Mean and S.E.M. of N=5-6 rats. (*) indicates a significant increase in RCLDW compared to RCLDW values of Phosgene alone exposed animals, ($p \leq 0.05$). Error bars above the cross-hatch region indicate a significant elevation of RCLDW values compared to values from animals exposed to air only, ($p \leq 0.05$).

exposed to the phosgene under "normal" V_E conditions are summarized in Figure 27. In this study, V_E was increased during the phosgene exposures by the concurrent delivery of 5% CO_2 with the test atmospheres. Although average protein concentrations in BALF appeared to be somewhat higher in samples obtained from rats that inhaled the phosgene along with CO_2 compared to their phosgene exposed control counterparts, such elevations were not significant ($P>0.05$).

The effects of increasing V_E during exposure to 13.9 ppm phosgene relative to BALF LDH activities are shown in Figure 28. As of 1 and 3 hrs after exposure, the co-inhalation of phosgene and CO_2 did not produce any demonstable increases in LDH activities in the BALF beyond those obtained with rats that were exposed to the phosgene under "normal" V_E conditions.

Increased V_E During Phosgene Exposure and BALF Cellular Changes: The effects of CO_2-induced increases in V_E during exposure to 13.9 ppm phosgene are summarized in Table 13. These lavage samples were obtained 1 and 3 hrs after the phosgene or phosgene + CO_2 exposures. Phosgene + CO_2 caused a near doubling of the numbers of lavageable alveolar macrophages (AM) as of 1 hr after exposure. However, as of 3 hrs after exposure, the numbers of AM harvested from the phosgene + CO_2 exposed lungs were less than half of the numbers of AM lavaged from the lungs of rats exposed to phosgene only. In conjunction with previously discussed lavaged cell findings, the lavaged AM number data obtained in this component of the study also suggest that phosgene and phosgene + CO_2 may exert an effect on the adherence characteristic of the AM *in situ*. No evidence was obtained in this study for an enhanced rate of recruitment of PMN into the alveoli due to the inhalation of the phosgene at a higher V_E.

Post-Exposure Potentiation of Phosgene-Induced Lung Injury

Lung Gravimetric Changes: The data summarized in Figures 29 and 30 represent the post-exposure LWW and RCLDW measurements from animals that were exposed to 9.3 ppm phosgene for 10 min and rested or subjected to a single exercise bout at various times following exposure. While the overall trend of measured LWW, Figure 29, was for increases in those animals exercised after exposure compared to exposed and rested controls, no significant difference in LWW occurred if animals were exercised at any time after exposure to this concentration of phosgene compared to values obtained on animals exposed to 9.3 ppm phosgene and rested after exposure. RCLDW measurements, Figure

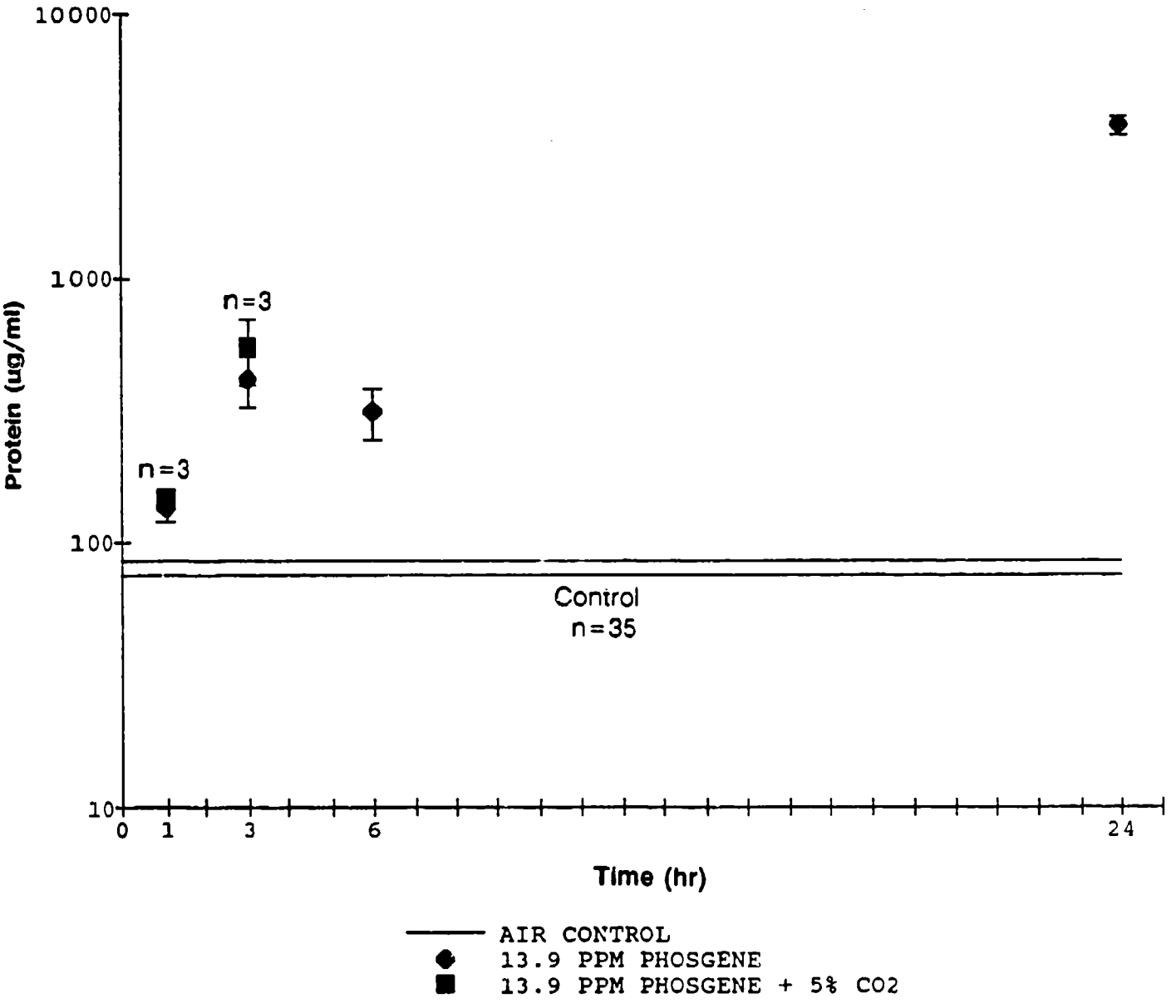

Figure 27 Effects of the co-inhalation of 13.9 ppm phosgene and 5% CO_2 on protein concentrations in lavage fluids. While average protein concentrations appeared to be further elevated in lavage fluids from animals exposed to phosgene and CO_2, values obtained from these animals were not significantly different from those protein concentrations measured in the lavage fluids of rats that were exposed to phosgene only.

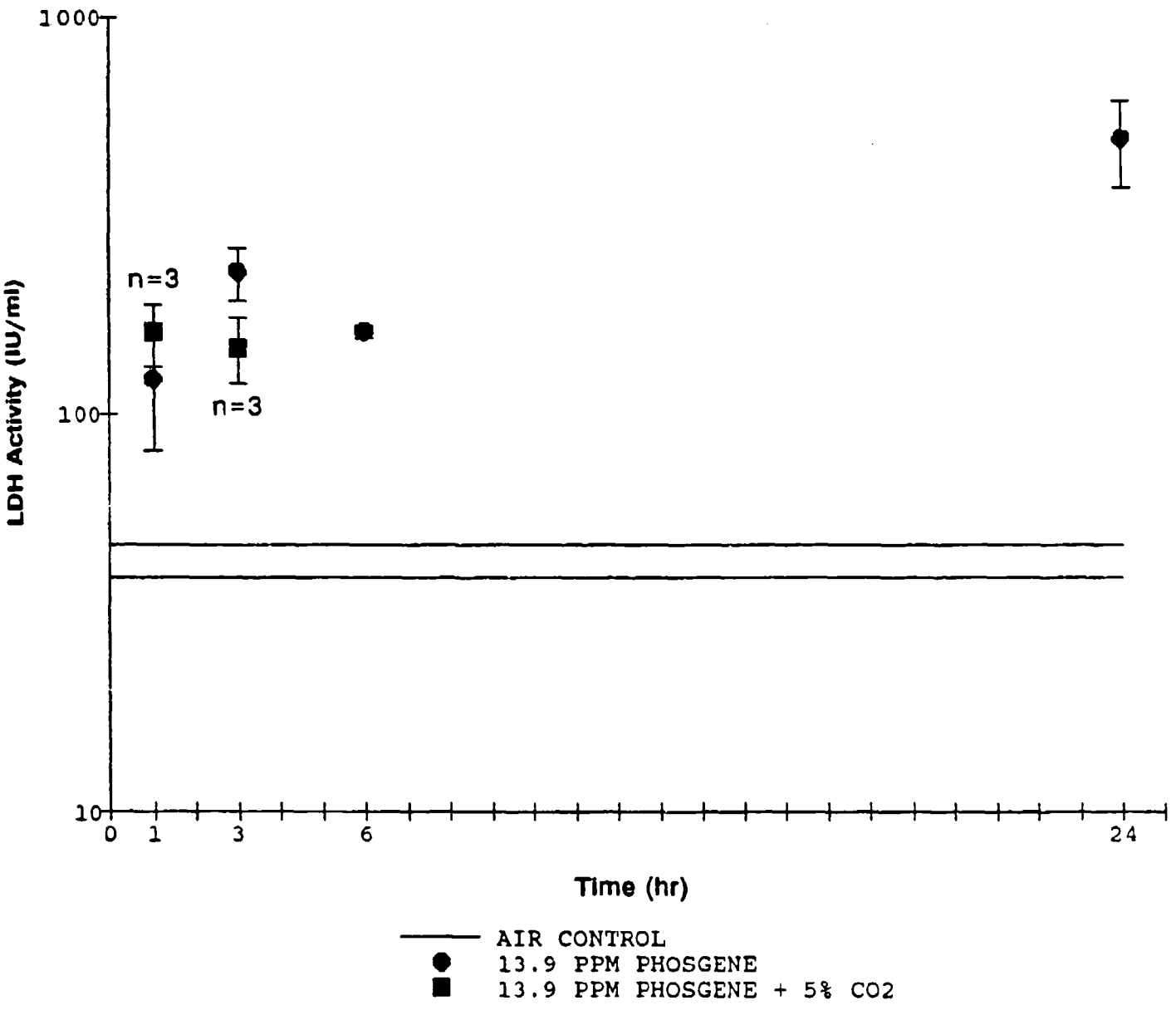

Figure 28 Effects of the co-inhalation of 13.9 ppm phosgene and 5% CO_2 on LDH activity in lavage fluids. No significant differences w· ·e found between the LDH activity in the lavage fluids of phosgene exposed and phosgene and CO_2 exposed rats as of 3 hours following the exposure.

Table 13 Numbers ($x10^{-7}$) of Different Cell Types Lavaged at Various Times after Exposure to 13.9 ppm Phosgene or 13.9 ppm Phosgene + 5% CO_2

Phosgene Only

1 hr Post-Exposure

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
0.882±0.095	0.872±0.093	0.003±0.002	0.005±0.002	0.002±0.001	0.001±0.001	6

3 hrs Post-Exposure

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.373±0.124	1.348±0.123	0.009±0.003	0.007±0.001	0.008±0.003	0.002±0.002	3

Phosgene + 5% CO_2

1 hr Post-Exposure

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.923±0.219*	1.906±0.210*	0.005±0.005	0.005±0.005	0.008±0.008	0	3

3 hrs Post-Exposure

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
0.659±0.092**	0.642±0.093**	0.006±0.001	0.003±0.001	0.007±0.001	0	3

Total Cells: all lavaged cells; **AM**: alveolar macrophages; **PMN**: polymorphonuclear leukocytes; **Lymph**: lymphocytes; **Mono**: monocytes/small mononuclear cells; **Eos**: eosinophils; **n**: numbers of animals studied. *: significantly higher than corresponding values obtained with phosgene only ($p<0.05$). **: significantly lower than corresponding values obtained with phosgene only ($p<0.05$).

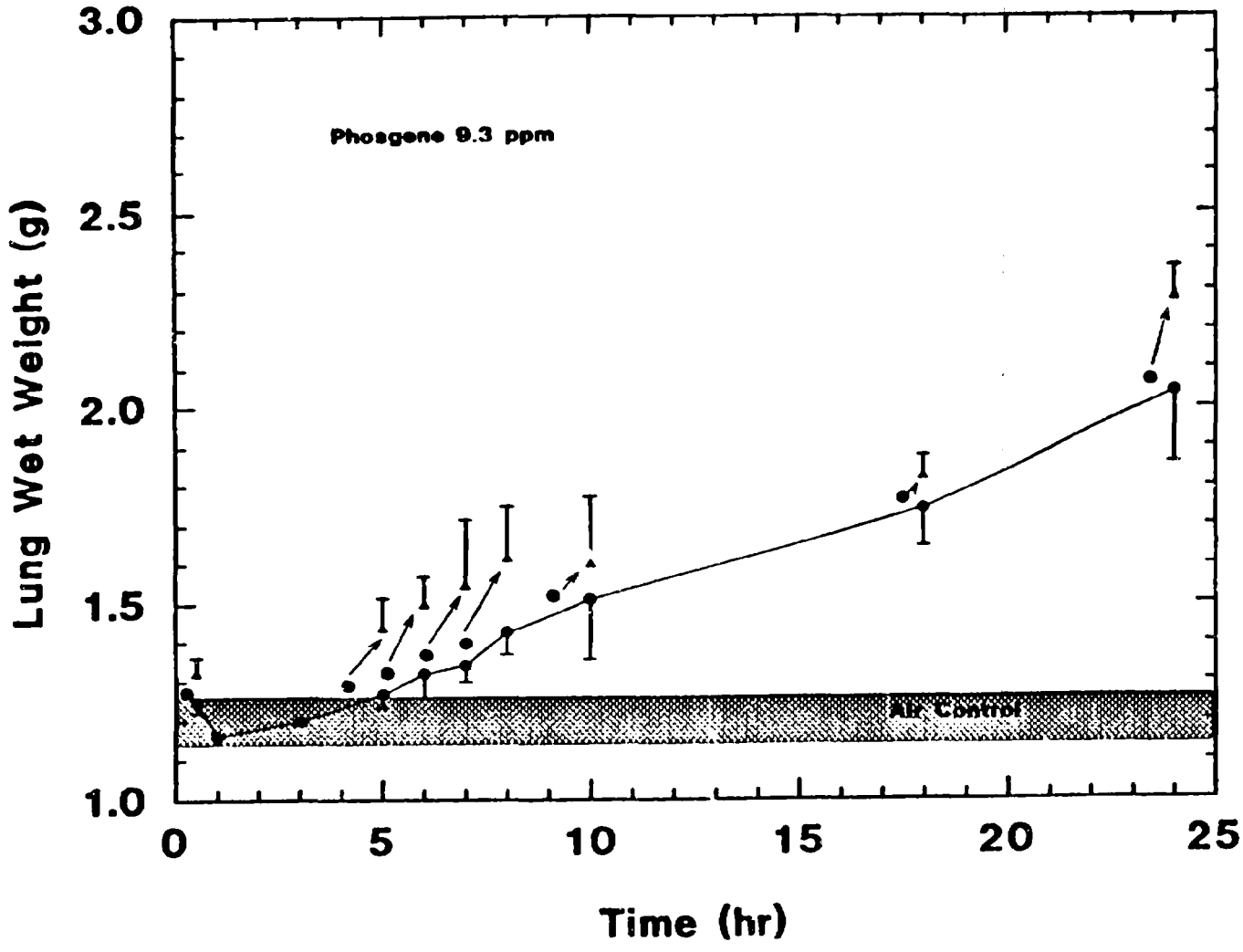

Figure 29 Lung Wet Weight (LWW) responses (closed circles) measured on groups of animals exposed to 9.3 ppm Phosgene for a 10 minute duration, rested after exposure, and sacrificed at various times after exposure. (e) represents the time seperate groups of animals were exercised after being exposed to 9.3 ppm Phosgene for 10 minutes and solid triangles represent the resulting LWW values measured on the exercised animals. No significant elevation of LWW values occur in animals exercised at any time point after exposure. Each point represents the Mean and S.E.M. of N=5 or more animals. Error bars above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, ($p \leq 0.05$).

30, also suggested that exercise after exposure to 9.3 ppm phosgene had no effect on the outcome of the injury when the exercise was performed within hours after exposure. However, if exercise was performed 23 hrs after exposure to 9.3 ppm phosgene, RCLDW was significantly elevated (~14%) as of 24 hrs after exposure compared to RCLDW values measured on animals exposed to this concentration of phosgene and rested after exposure. As well, exercise immediately after exposure to 9.3 ppm phosgene had a long term effect on phosgene-induced lung injury. Specifically, the LWW, Figure 31, and RCLDW values, Figure 32, values obtained with animals that were exposed to this concentration of phosgene, exercised immediately post-exposure, and sacrificed 24 hrs after exposure were significantly elevated (~15% for LWW and ~16% for RCLDW) compared to values obtained from animals exposed in like fashion but rested until they were sacrificed 24 hrs after the exposure.

Exercise performed after exposure to a higher concentration of phosgene gave somewhat different responses. Figures 33 and 34 show the LWW and RCLDW responses, respectively,measured with the lungs of animals exposed to 13.9 ppm phosgene and either rested after exposure or exercised at various times after exposure. In all cases, a single exercise bout performed after exposure to this concentration of phosgene resulted in significantly greater LWW and RCLDW values when animals were sacrificed shortly after the exercise bouts compared to rested control values.

Effects of Post-Exposure Exercise on BALF Protein and LDH Activities: The effects of post-exposure exercise on lavageable protein concentrations following exposure to 9.3 ppm and 13.9 ppm phosgene are summarized in Figures 35 and 36, respectively. As indicated in Figure 35, the performance of exercise immediately after exposure to 9.3 ppm phosgene had no significant effect on the concentration of protein in the lavage fluids of animals sacrificed 24 hrs after the exposure relative to protein values obtained with phosgene exposed and rested rats. Later exercise performed 23 hrs after exposure to the 9.3 ppm concentration of phosgene also did not increase BALF protein in rats that were sacrificed 1 hr after the exercise bout.

Exercise performed immediately after exposure to the 13.9 ppm concentration of phosgene, Figure 36, resulted in a significant increase in BALF protein as of 1 hr after exposure ($P<0.003$). Like with the 9.3 ppm phosgene concentration, exercise performed 23 hrs after exposure to 13.9 ppm phosgene caused no further increases in BALF protein beyond values obtained from rats that were identically exposed to the phosgene and allowed to rest prior to being sacrificed 24 hrs after exposure.

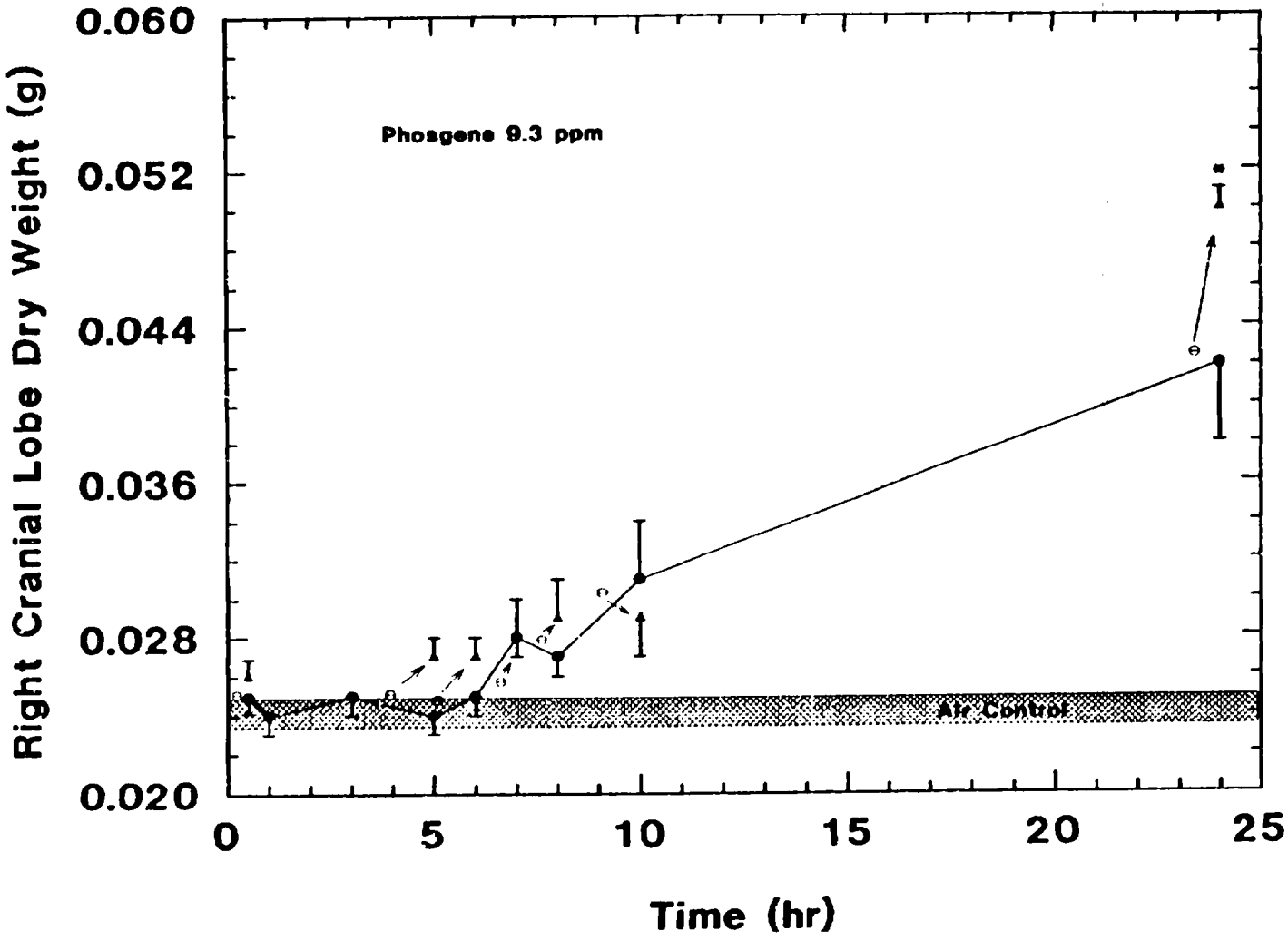

Figure 30 Right Cranial Lobe Dry Weight (RCLDW) responses (closed circles) measured on groups of animals exposed to 9.3 ppm Phosgene for a 10 minute duration, rested after exposure, and sacrificed at various times after exposure. (e) represents the time seperate groups of animals were exercised after being exposed to 9.3 ppm Phosgene for 10 minutes and solid triangles represent the resulting RCLDW values measured on the exercised animals. No significant elevation of RCLDW values occur in animals exercised at any time point after exposure. Each point represents the Mean and S.E.M. of N=5 or more animals. Error bars above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, ($p \leq 0.05$).

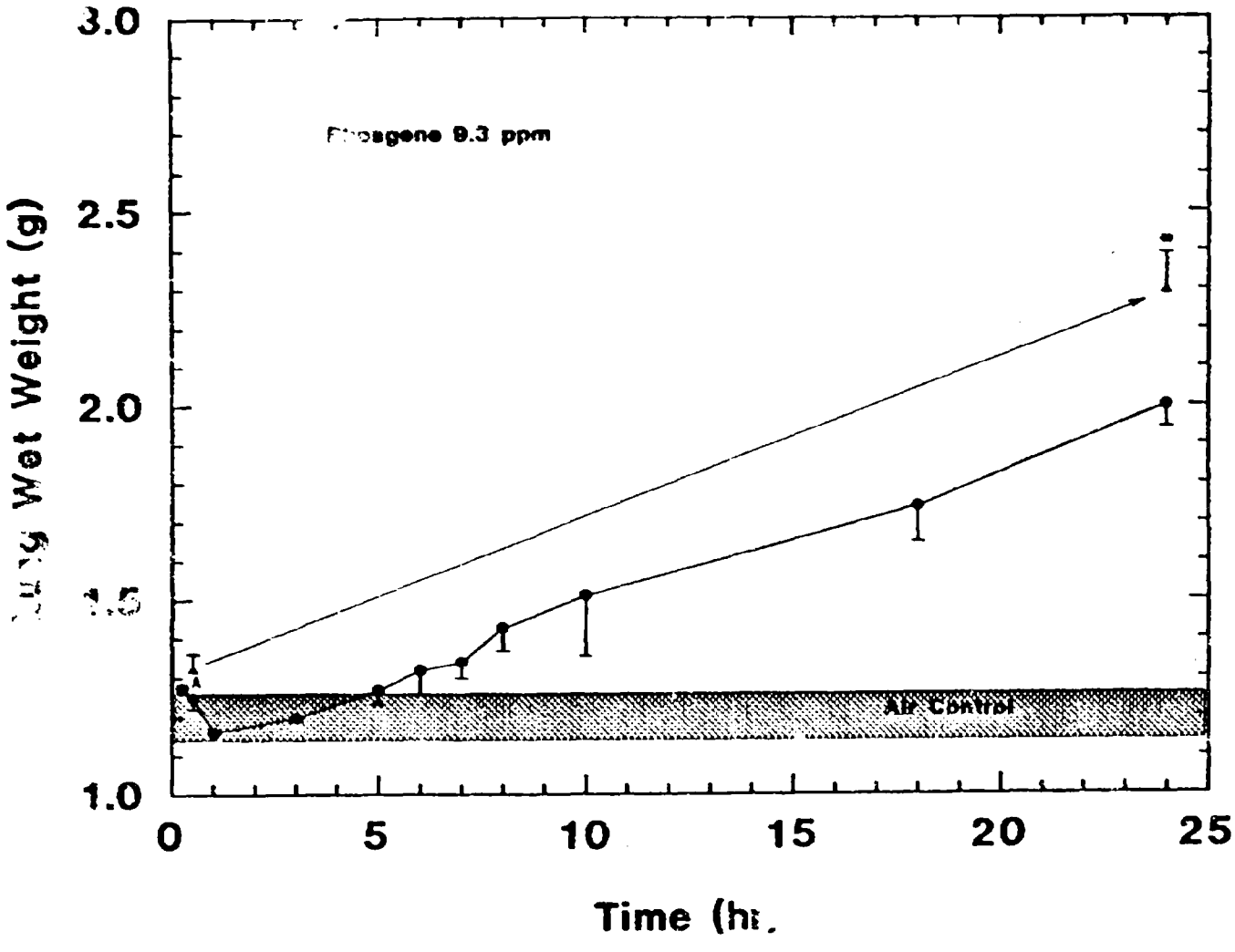

Figure 31 Lung Wet Weight (LWW) responses (closed circles) measured on groups of animals exposed to 9.3 ppm Phosgene for a 10 minute duration, rested after exposure, and sacrificed at various times after exposure. (e) represents the time a seperate group of animals were exercised after being exposed to 9.3 ppm Phosgene for 10 minutes and solid triangles represent the resulting LWW values measured on the exercised animals. (*) indicates a significant elevation of LWW values compared to values measured on Phosgene exposed and rested animals, ($p \leq 0.05$). Each point represents the Mean and S.E.M. of N = 5 or more animals. Error bars above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, ($p \leq 0.05$).

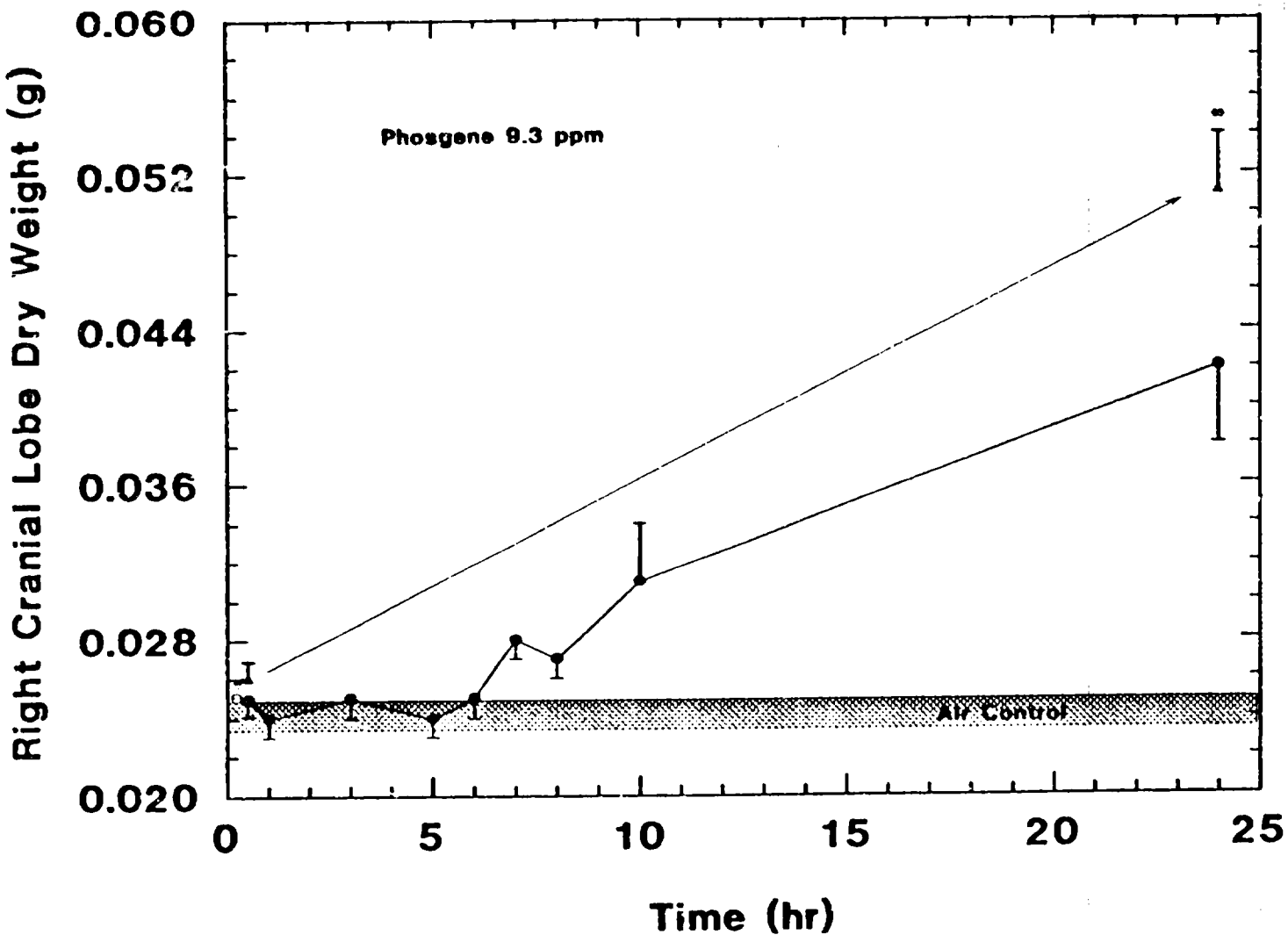

Figure 32 Right Cranial Lobe Dry Weight (RCLDW) responses (closed circles) measured on groups of animals exposed to 9.3 ppm Phosgene for a 10 minute d[illegible]ion, rested after exposure, and sacrificed at various times after exposure. (e) represents the time a seperate group of animals were exer[illegible]er being exposed to 9.3 ppm Phosgene for 10 minutes and solid triangles represent the resulting RCLDW values measured on the exerci[illegible]mals (*) indicates a significant elevation of RCLDW values compared to values measured on Phosgene exposed and rested animals, ($p \leq$ [illegible]oint represents the Mean and S.E.M. of N=5 or more animals. Error bars above the cross-hatch region indicate significant elevation of mea[illegible]alues compared to air exposed controls, ($p \leq 0.05$).

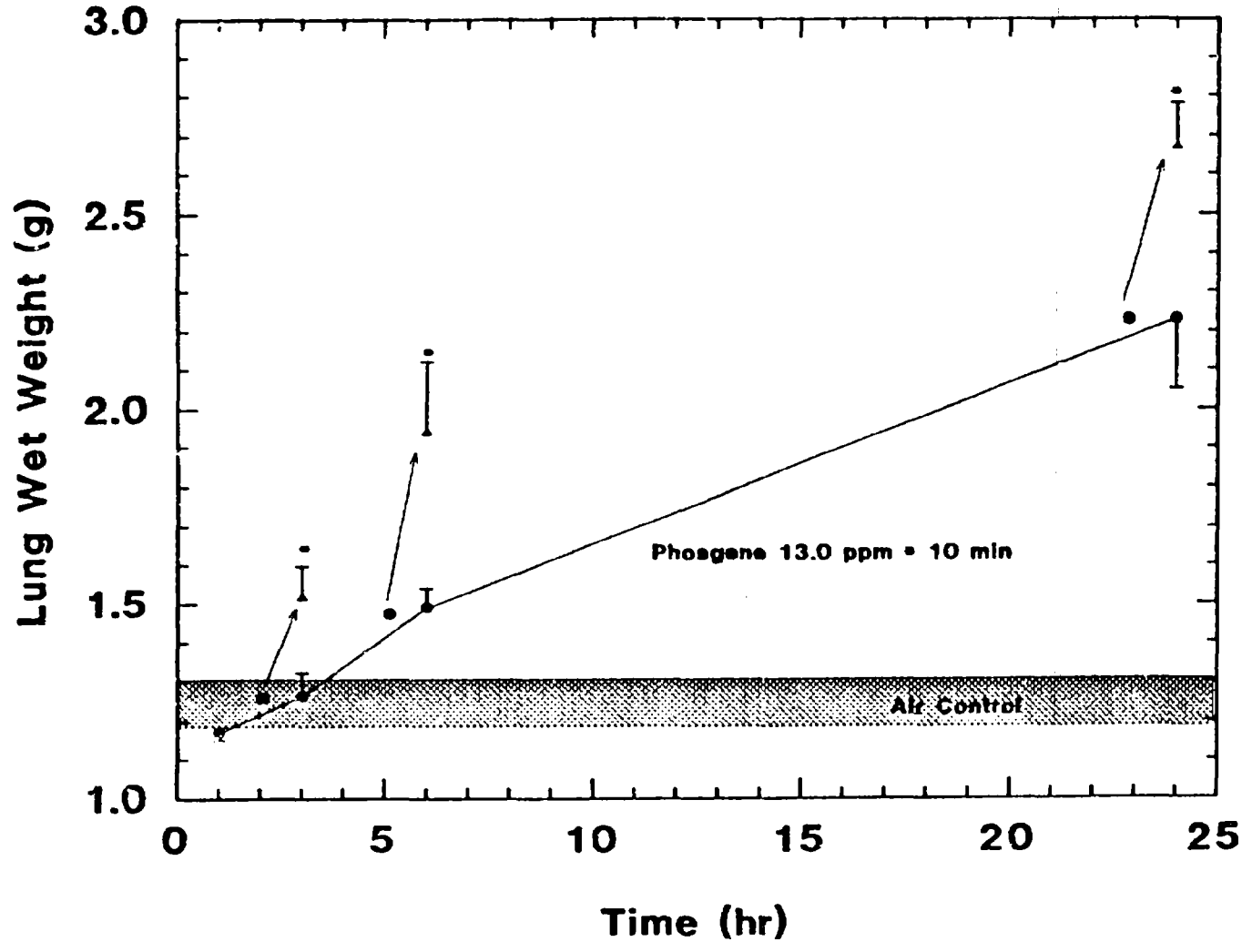

Figure 33 Lung Wet Weight (LWW) responses (closed circles) measured on groups of animals exposed to 13.9 ppm Phosgene for a 10 minute duration, rested after exposure, and sacrificed at various times after exposure. (o) represents the time seperate groups of animals were exercised after being exposed to Phosgene for 10 minutes and solid triangles represent the resulting LWW values measured on the exercised animals. Each point represents the Mean and S.E.M. of N=5 or more animals. Error bars above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, ($p \leq 0.05$).

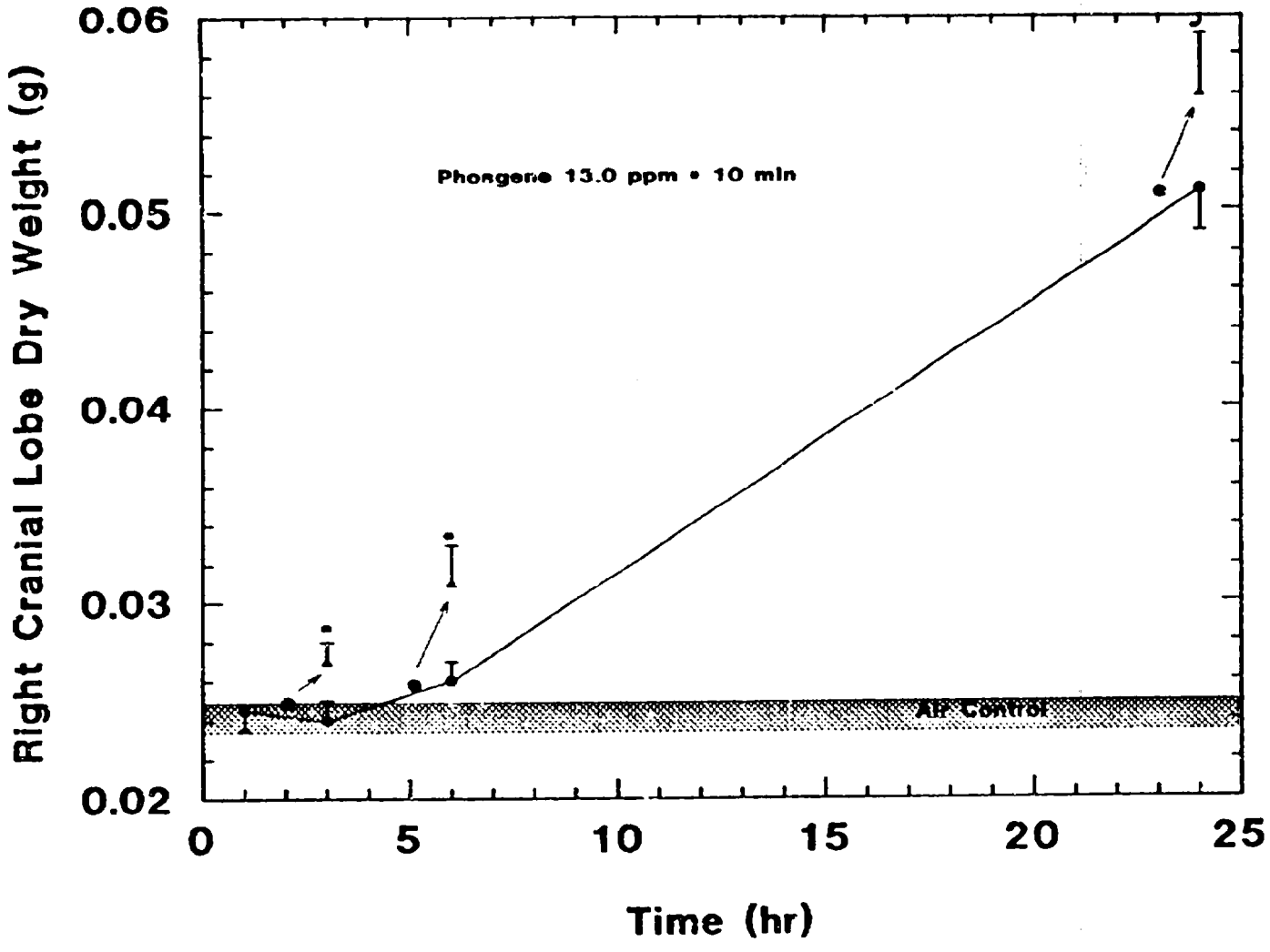

Figure 34 Right Cranial Lobe Dry Weight (RCLDW) responses (closed circles) measured on groups of animals exposed to 13.9 ppm Phosgene for a 10 minute duration, rested after exposure, and sacrificed at various times after exposure. (e) represents the time seperate groups of animals were exercised after being exposed to 9.3 ppm Phosgene for 10 minutes and solid triangles represent the resulting RCLDW values measured on the exercised animals. Each point represents the Mean and S.E.M. of N=5 or more animals. Error bars above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, ($p \leq 0.05$).

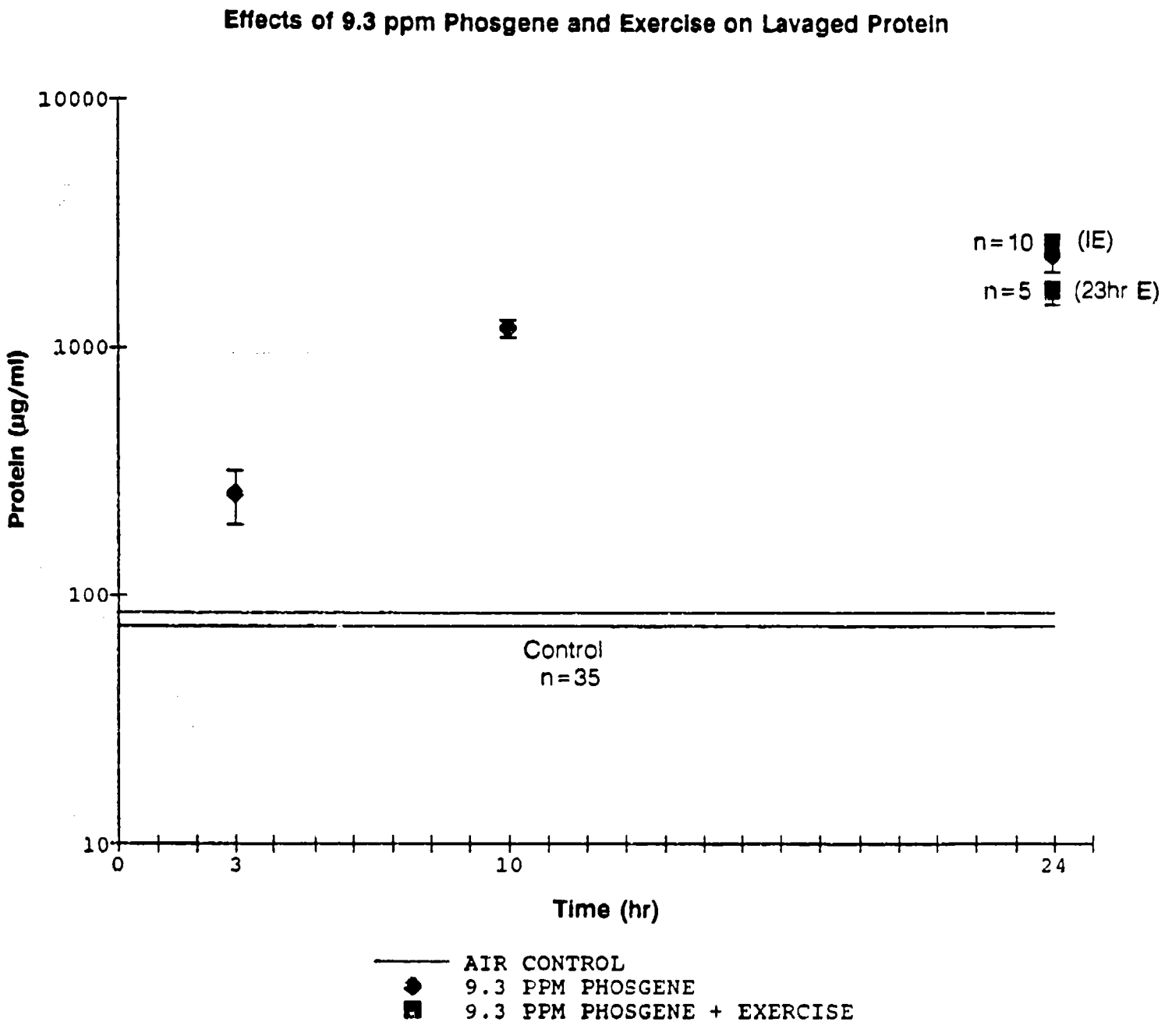

Figure 35 Effects of post-exposure exercise on protein concentrations in lavage fluids. Exercise performed immediately after exposure (IE) or 23 hours after exposure (23hr E) to 9.3 ppm phosgene did not significantly affect lavaged protein concentration compared to protein values measured in the lavage fluids of rats that were exposed to the 9.3 ppm concentration of phosgene and allowed to rest until the 24 hour post-exposure sacrifice time.

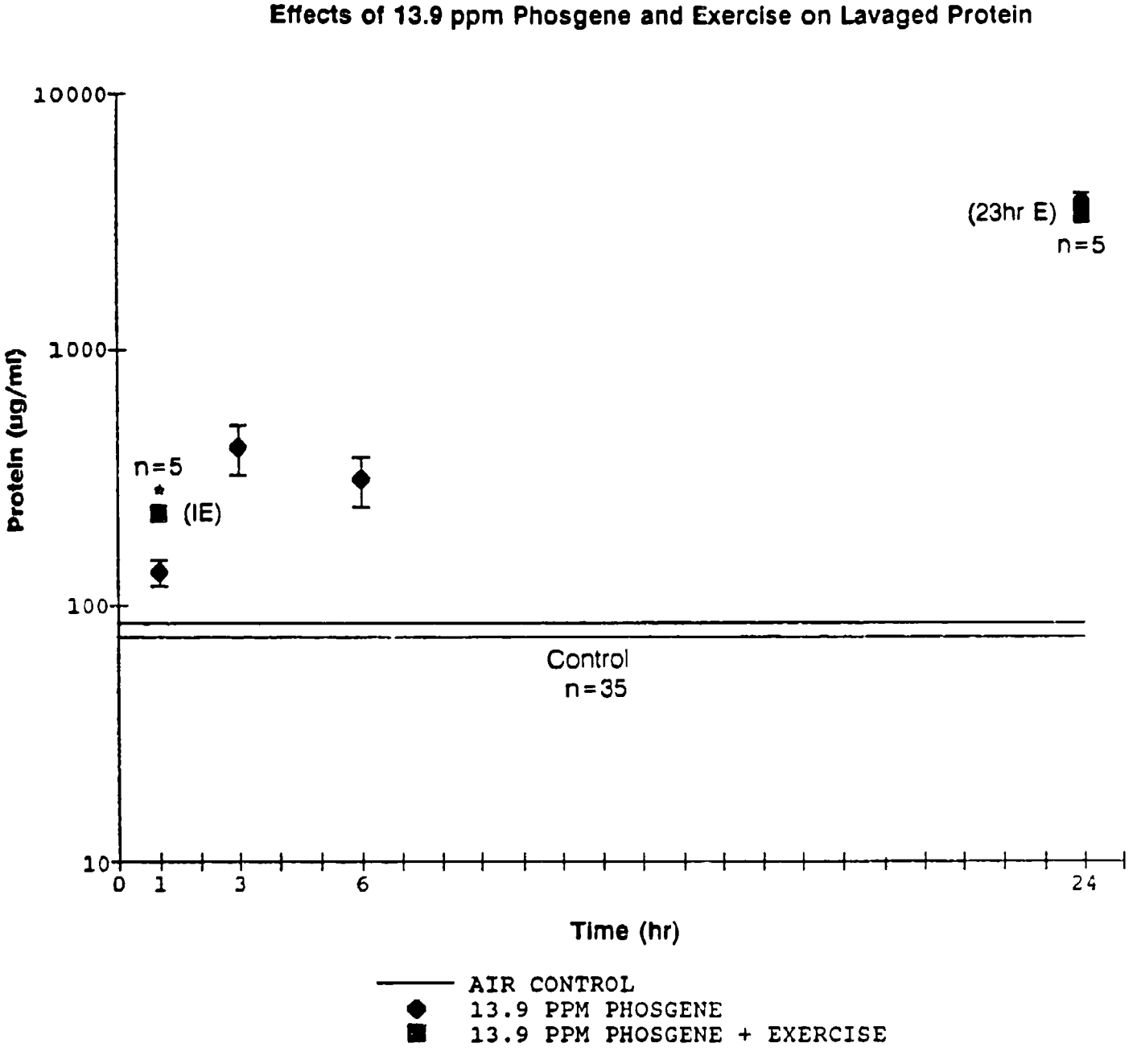

Figure 36 Effects of post-exposure exercise on protein concentrations in lavage fluids. Exercise performed immediately after exposure to 13.9 ppm phosgene (IE) resulted in a significant increase ($p < 0.003$) in the lavage fluids of rats that were sacrificed shortly after exposure and exercise (*). Exercise performed as of 23 hours after exposure (23hr E) had no significant effects on lavageable protein.

The effects of post-exposure exercise on BALF LDH activities following exposure to the two concentrations of phosgene are summarized in Figures 37 and 38. Exercise performed immediately after 9.3 ppm phosgene exposure did not result in any further increases in LDH activities in BALF of rats sacrificed 24 hrs after the exposure compared to LDH activity values obtained from rats that were exposed to the phosgene and allowed to rest until being sacrificed at the same corresponding post-exposure time point, Figure 37. When exercise was performed 23 hrs after exposure to the 9.3 ppm concentration of phosgene, BALF LDH activities measured 1 hr later were actually significantly less (P=0.05) than those measured in BALF of corresponding phosgene-exposed and rested control animals.

With the 13.9 ppm concentration of phosgene, one group of animals was exercise immediately after exposure and sacrificed 1 hr after the exposure for the BALF LDH activity studies, Figure 38. No evidence was obtained to suggest that early post-exposure exercise in itself potentiates the cell cytotoxicity of phosgene. As well, no evidence was found to suggest that exercise performed 23 hrs after exposure to the 13.9 ppm concentration of phosgene further promoted cell injury in that the LDH activites in the BALF from the exercised rats were very similar to those obtained from phosgene-exposed rats that were allowed to rest until being sacrificed for the BALF studies at the corresponding sacrifice time.

Post-exposure Exercise and BALF Cellular Changes: The lavaged cell data obtained after rats were exposed to 9.3 ppm phosgene and either exercised or rested following exposure are summarized in Table 14. Exercise performed immediately or 23 hrs after exposure had no significant effect on the numbers of lavageable AM, PMN, or blood monocytes as of 24 hrs after exposure.

The lavaged cell data obtained after rats were exposed to the higher 13.9 ppm concentration of phosgene and either rested or exercised after the exposures are summarized in Table 15. In this set of experiments, the percentages of each lavaged cell type are compared in that cell counts were not obtained. Compared to the post-phosgene exposure-rested condition, exercise performed immediately or 23 hrs after exposure to 13.9 ppm phosgene had no detectable effect on the percentages of the various cell types as of 24 hrs after the exposures.

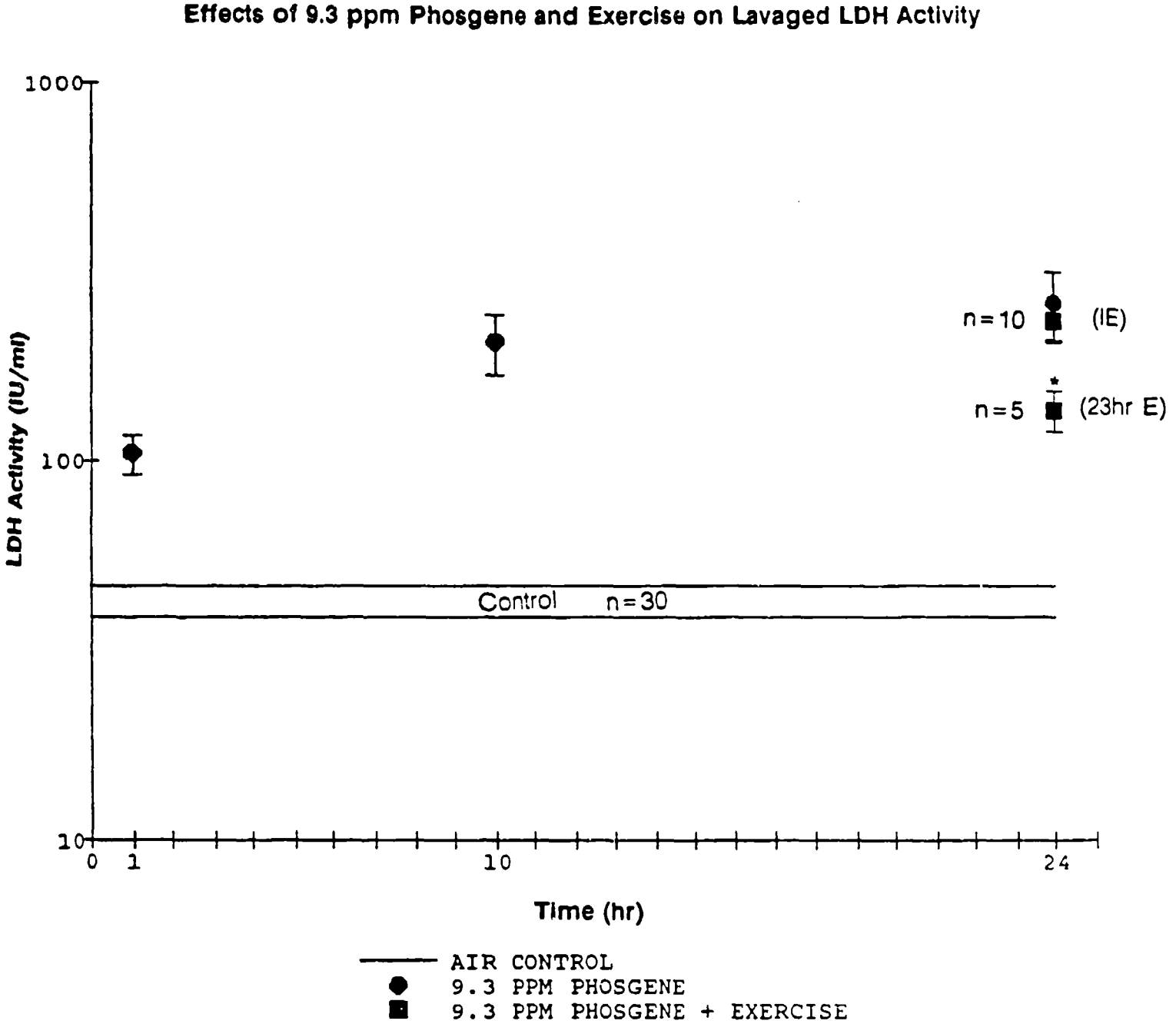

Figure 37 Effects of post-exposure exercise on LDH activity in lavage fluids following exposure to 9.3 ppm phosgene. Exercise performed immediately after exposure (IE) did not significantly affect LDH activity present in lavage fluids at the 24 hour post-exposure sacrifice time point. However, exercise performed 1 hour before the 24 hour sacrifice time (23hr E) resulted in a significant reduction (*) in lavageable LDH activity ($p \leq 0.05$).

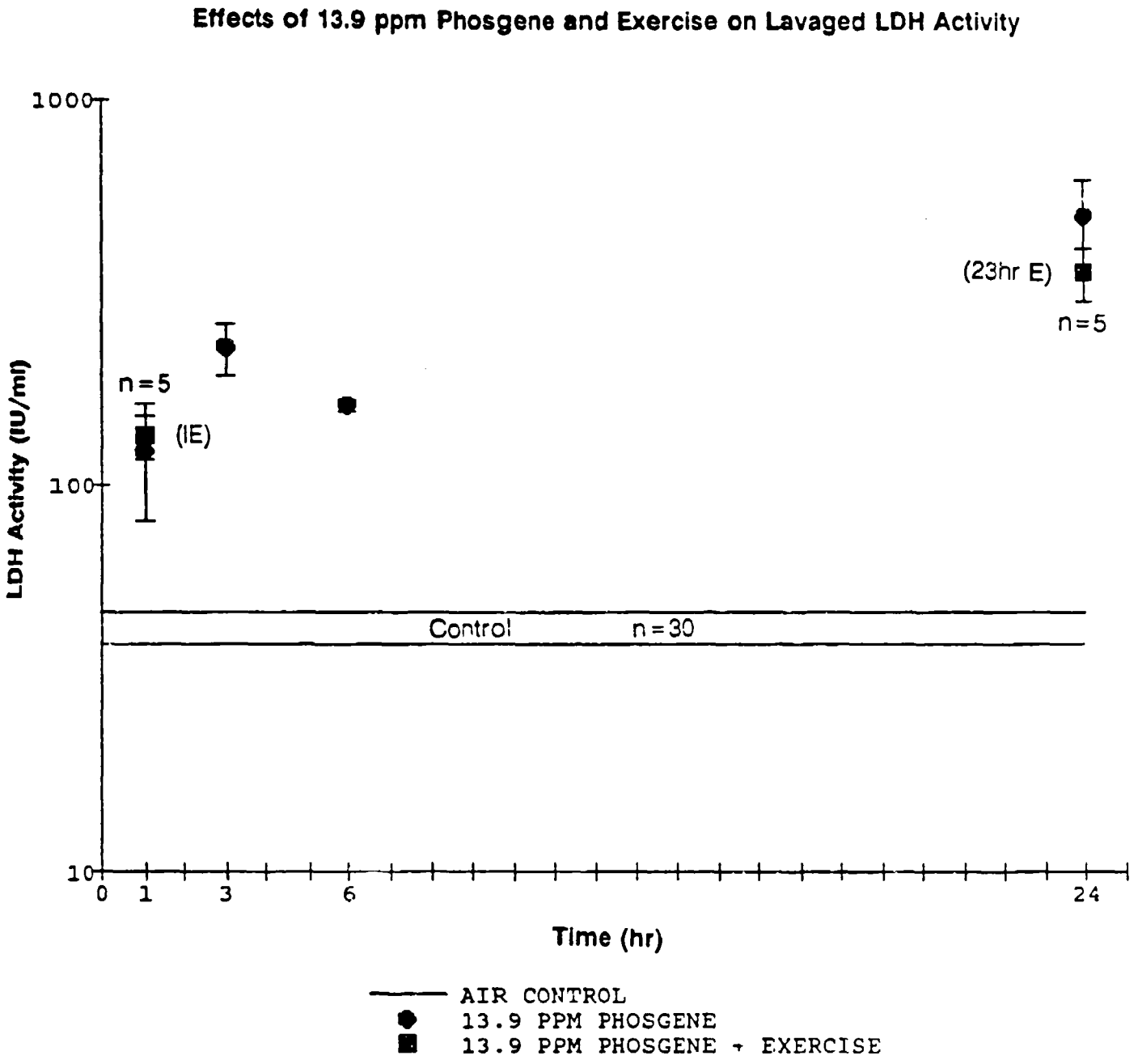

Figure 38 Effects of post-exposure exercise on LDH activity in lavage fluids following exposure to 13.9 ppm phosgene. Exercise performed immediately after exposure (IE) to the phosgene did not significantly increase LDH activity in the lavage fluids of rats that were sacrificed shortly thereafter. Likewise, exercise performed 23 hours after exposure (23hr E) did not significantly increase LDH in the lavage fluids of rats sacrificed 1 hour after the exercise bout.

Table 14 Lung Free Cell Numbers following 9.3 ppm Phosgene Exposure and Post-Exposure Exercise Compared to Numbers Harvested from Rats that Were Exposed to 9.3 ppm Phosgene and Then Rested until Bronchoalveolar Lavage

Exposed and Rested Rats

24 hrs Post-Exposure

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.537±0.134	1.052±0.085	0.238±0.039	0.116±0.027	0.130±0.025	0.001±0.001	10

Exposed and Exercised Rats

Immediate Exercise, 24 hr Sacrifice

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.484±0.164	0.941±0.111	0.341±0.073	0.076±0.021	0.124±0.023	0.002±0.001	5

Exercise at 23 hrs Post-Exposure, 24 hr Sacrifice

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.394±0.074	1.029±0.081	0.171±0.028	0.138±0.015	0.055±0.028	0.001±0.001	5

Total Cells: all lavaged cells; **AM**: alveolar macrophages; **PMN**: polymorphonuclear leukocytes; **Lymph**: lymphocytes; **Mono**: monocytes/small mononuclear cells; **Eos**: eosinophils; **n**: numbers of animals studied. *: significantly higher than corresponding rested control values ($p<0.05$). **: significantly lower than corresponding rested control values ($p<0.05$).

Table 15 Lung Free Cell Numbers ($x10^{-7}$) or Percentages following 13.9 ppm Phosgene Exposure and Post-Exposure Exercise Compared to Numbers or Percentages Harvested from Rats that Were Exposed to 13.9 ppm Phosgene and then Rested until Bronchoalveolar Lavage

Exposed and Rested Rats

1 hr Post-Exposure ($x10^{-7}$)

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
0.882±0.095	0.872±0.093	0.003±0.002	0.005±0.002	0.002±0.001	0.001±0.001	6

24 hrs Post-Exposures (%)

AM	PMN	Lymph	Mono	Eos	(n)
44.0±4.9	30.9±3.8	9.8±1.1	15.1±1.6	0.2±0.1	5

Exposed and Exercised Rats

Immediate Exercise, 1 hr Sacrifice ($x10^{-7}$)

Total Cells	AM	PMN	Lymph	Mono	Eos	(n)
1.146±0.100	1.134±0.100	0.008±0.002	0.001±0.001	0.003±0.002	0	4

Exercise at 23 hrs Post-Exposure, 24 hr Sacrifice (%)

AM	PMN	Lymph	Mono	Eos	(n)
46.7±4.4	28.9±3.0	6.9±0.8	16.9±1.4	0.4±0.1	5

Total Cells: all lavaged cells; **AM**: alveolar macrophages; **PMN**: polymorphonuclear leukocytes; **Lymph**: lymphocytes; **Mono**: monocytes/small mononuclear cells; **Eos**: eosinophils; **n**: numbers of animals studied. *: significantly higher than corresponding rested control values ($p<0.05$). **: significantly lower than corresponding rested control values ($p<0.05$).

Exercise Potentiation of Injury Following the Inhalation of Phosgene at Elevated Minute Ventilation: In this study component, we focused on how the performance of exercise following the inhalation of phosgene concurrently with 5% CO_2 to elevate minute ventilation) might affect the injurious response. The 9.3 ppm phosgene level was selected for these experiments inasmuch as: 1) a 10 min exposure to this concentration gives an ~6-7 hr post-exposure latency period, as defined by lung gravimetric criteria, 2) exercise performed after exposure to 9.3 ppm phosgene generally does not result in a significant potentiation of the injurious response, 3) 9.3 ppm + 5% CO_2 results in a shortening of the latency period to 3-4 hrs. and 4) 9.3 ppm + 5% CO_2 results in a more pronounced pulmonary edematous response than when 9.3 ppm phosgene is inhaled alone, with the limits of both injurious responses being within a range where any potentiating effects of post-exposure exercise could be readily resolved by gravimetric increases.

As previously indicated, exercise performed following exposure to the 9.3 ppm concentration of phosgene generally did not significantly potentiate the injurious response, refer to Figure 29. As illustrated by the lung gravimetric data summarized in Figure 39 and 40, however, exercise did potentiate the injurious response when the phosgene was inhaled during CO_2-induced increases in minute ventilation. Even during the shortened latency period, exercise resulted in significant increases in LWW, Figure 39, and generally significant increases in the LWW parameter occurred when exercise was performed by the phosgene + CO_2 exposed rats at later post-exposure times relative to LWW changes that were obtained with phosgene + CO_2 exposed but rested rats. With the RCLDW parameter, Figure 40, no significant exercise-associated changes were found when exercise was performed dueing the latency period following phosgene + CO_2 exposure, but at later post-exposure exercise times, i.e., 7 and 23 hrs, RCLDW increases paralleled the increases in LWW.

The histopathologic data from this study are summarized in Tables 16a and 16b. The histopathologic major finding was that exercise performed 2 hrs after exposure to 9.3 ppm phosgene + 5% CO_2 resulted in increases in alveolar fibrin as of 1 hr after the exposure. Otherwise, histologic changes that occurred in the lungs of the other exercised and rested groups of exposed rats were closedly similar.

Collectively, the above findings indicate that exercise performed during the latency period that may follow phosgene + CO_2 exposure can result in a potentiation of the injurious response, and exercise performed during a 23 hr post-exposure interval following exposure to phosgene + CO_2 can result in more pulmonary edema than the severity of the pulmonary edematous response that is already increased due to the inhalation of phosgene + CO_2 in the absence of exercise.

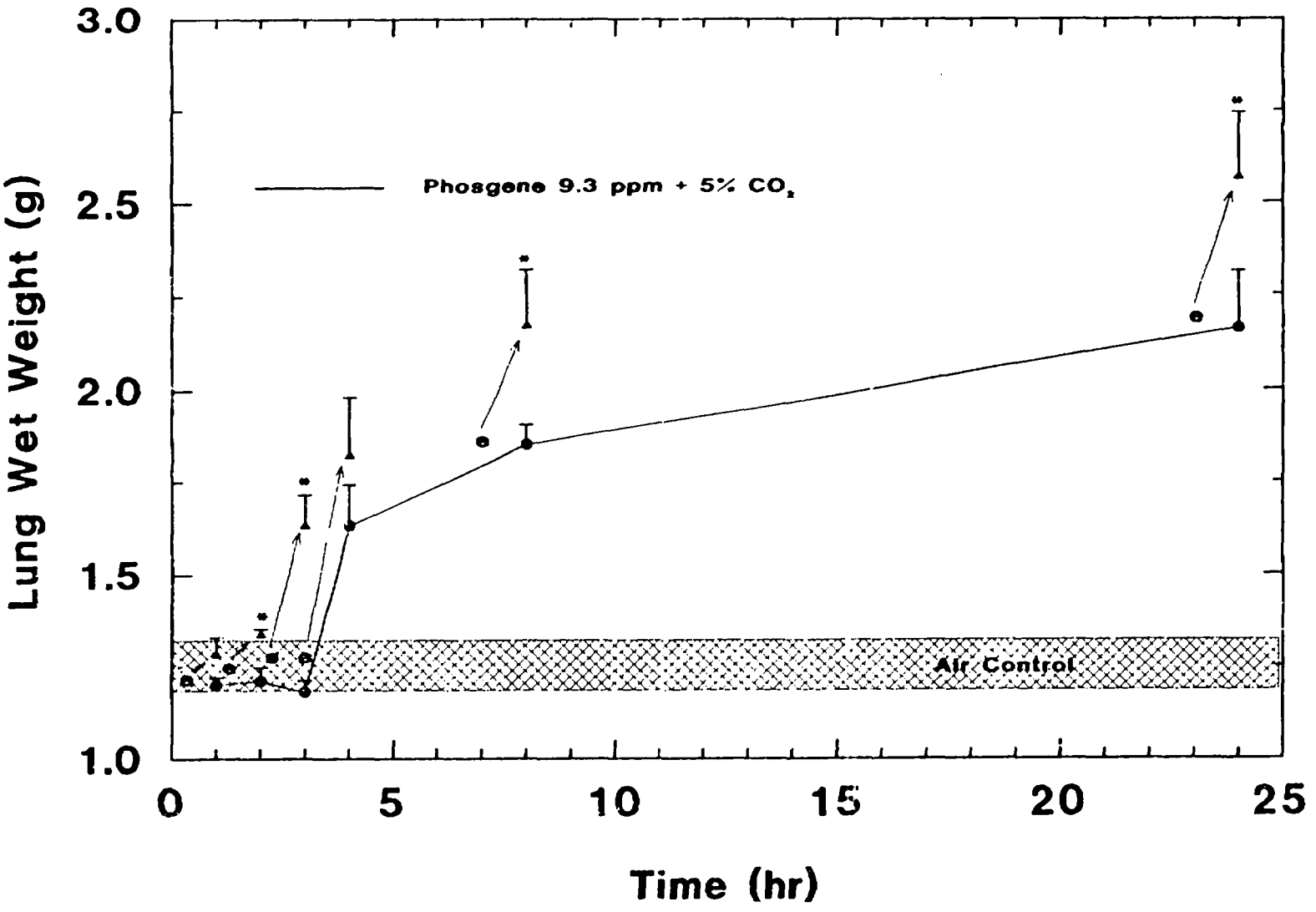

Figure 39 Lung wet weight (LWW) values of animals at various times after exposure to 9.3 ppm Phosgene + 5% CO_2. Solid circle and solid line indicate the LWW response of exposed animals followed by post-exposure rest. (e) indicates the time groups of rats were exercised after exposure. Solid triangle and arrows indicate the resulting LWW increase if animals are exercised at the indicated times. Each point represents the mean and S.E.M. of N = 5 or more animals. S.E.M. line above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, $P \leq 0.05$. (*) indicates a significant elevation of measured values on exercised rats compared to Phosgene exposed and rested values, $P \leq 0.05$.

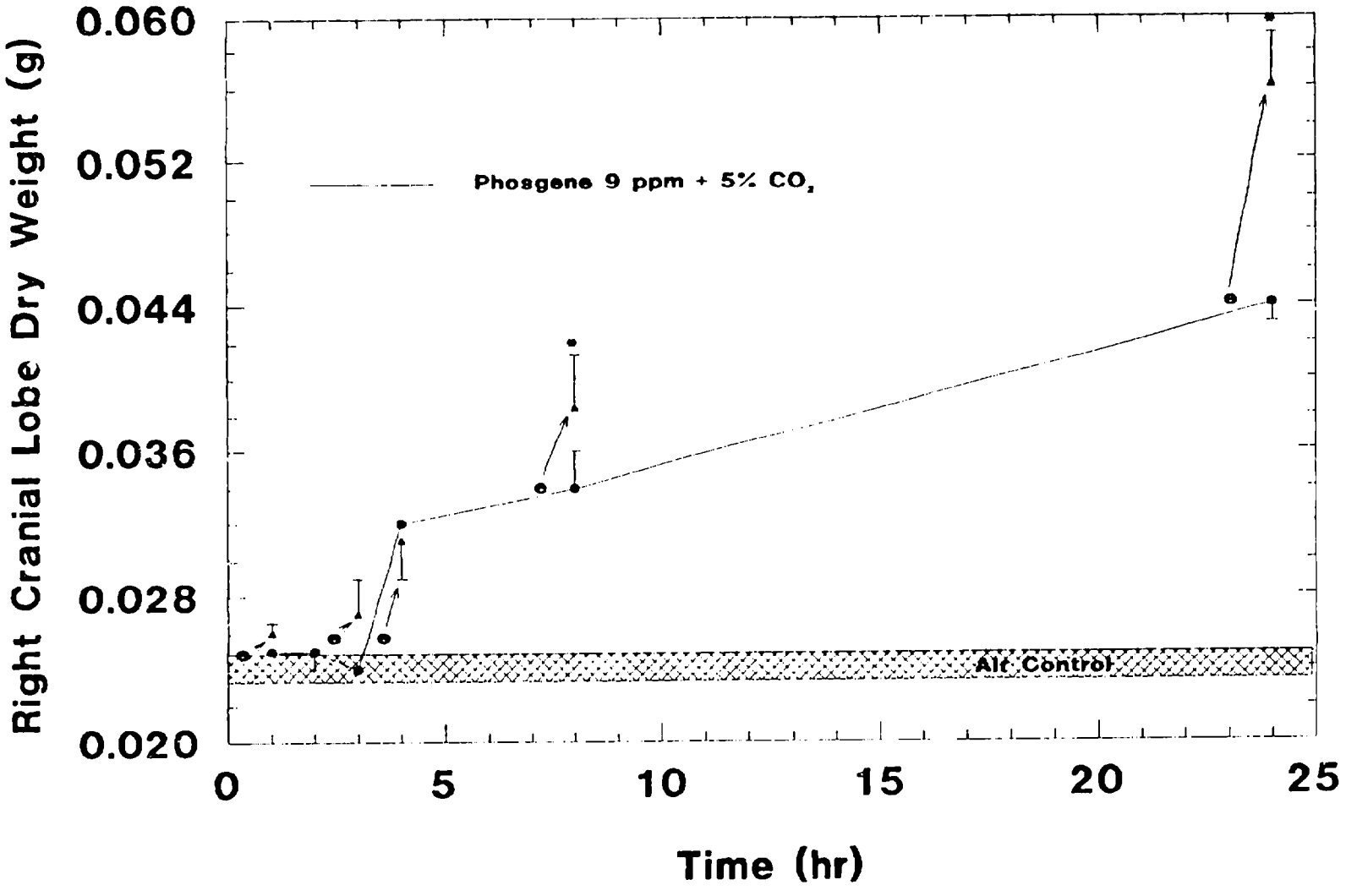

Figure 40 Right cranial lobe dry weight (RCLDW) values of animals at various times after exposure to 9.3 ppm Phosgene + 5% CO_2. Solid circle and solid line indicate the RCLDW response of exposed animals followed by post-exposure rest. (e) indicates the time groups of rats were exercised after exposure. Solid triangle and arrows indicate the resulting RCLDW increase if animals are exercised at the indicated times. Each point represents the mean and S.E.M. of N = 5 or more animals. S.E.M. line above the cross-hatch region indicate significant elevation of measured values compared to air exposed controls, $P \leq 0.05$. (*) indicates a significant elevation of measured values on exercised rats compared to Phosgene exposed and rested values, $P \leq 0.05$.

Exercise Time (hr)	Sacrifice Time (hr)	*Distribution*	*Severity*	*Intensity*
		Edema		
0	1	0	0	0
1	2	0	0	0
2	3	0	0	0
3	4	0.2 ± 0.2	0.4 ± 0.4	0.6 ± 0.6
7	8	0	0	0
23	24	0	0	0
		Fibrin		
0	1	0	0	0
1	2	0.8 ± 0.5	0.7 ± 0.4	0.4 ± 0.2
2	3	2.8 ± 0.3*	2.3 ± 0.1*	1.3 ± 0.1*
3	4	2.6 ± 0.4	2.0 ± 0.0	1.3 ± 0.1
7	8	4.0 ± 0.0	2.6 ± 0.2	1.6 ± 0.1
23	24	4.0 ± 0.0	2.9 ± 0.1	2.3 ± 0.1
		Polymorphonuclear Leukocytes		
0	1	0	0	0
1	2	0	0	0
2	3	1.0 ± 0.6	0.8 ± 0.5	0.9 ± 0.5
3	4	0	0	0
7	8	3.6 ± 0.2	1.8 ± 0.1	1.5 ± 0.2
23	24	4.0 ± 0.0	2.2 ± 0.1	2.4 ± 0.2
		Red Blood Cells		
0	1	0	0	0
1	2	0	0	0
2	3	0	0	0
3	4	0	0	0
7	8	0	0	0
23	24	0.8 ± 0.5	1.2 ± 0.7	0.8 ± 0.5

Table 16a Histopathologic changes in the lung at various times after exposure to 9.3 ppm phosgene + 5% CO_2 for 10 minutes (*) denotes significant differences between lungs of animals exercised after receiving 9.3 ppm phosgene + 5% CO_2 for 10 minutes and those rested before sacrifice at the same times, $p \leq 0.05$

Exercise Time (hr)	Sacrifice Time (hr)	*Distribution*	*Severity*	*Intensity*
		Macrophages		
0	1	0	0	0
1	2	0.4 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
2	3	1.8 ± 0.6	1.0 ± 0.4	0.8 ± 0.3
3	4	1.6 ± 0.4	0.8 ± 0.2	0.9 ± 0.2
7	8	3.0 ± 0.0	1.9 ± 0.1	1.1 ± 0.1
23	24	4.0 ± 0.0	1.8 ± 0.2	1.2 ± 0.2

Table 16b Histopathologic changes in the lung at various times after exposure to 9.3 ppm phosgene + 5% CO_2 for 10 minutes. There are no significant differences between lungs of animals exercised after receiving 9.3 ppm phosgene + 5% CO_2 for 10 minutes and those rested before sacrifice at the same times, $p \leq 0.05$.

Work Performance Incapacitation Following Phosgene Exposure

A stated task in the original Statement of work was to measure maximum oxygen consumption (VO_{2max}) following phosgene exposure in order to evaluate work performance incapicitation. Another objective was to evaluate potential relationships between the extent of pre-existing pulmonary edema induced by phosgene exposure and reductions in work performance capacity. These studies focused on VO_{2max} and lung wet weight increases that follow 10 min exposures to 9.3 ppm and 13.9 ppm phosgene.

The work performance capabilities of groups of rats at various times after exposure to 9.3 ppm phosgene, as well as the corresponding changes in LWW values obtained from separate groups of rats at post-exposure time corresponding to the times exercise was performed for the VO_{2max} measurements are summarized in Figure 41. As indicated, VO_{2max} values become progressively reduced over time following exposure to the 9.3 ppm concentration of phosgene, with the observed maximal reduction (~28%) occurring as of 24 hrs after exposure. The progressive reduction of work performance capacity reflected the progressive increase in LWW values measured after exposure to this concentration of phosgene.

The work performance capabilities of groups of rats at various times after exposure to 13.9 ppm phosgene, as well as the corresponding changes in LWW values obtained from separate groups of rats at post-exposure time corresponding to the times exercise was performed for the VO_{2max} measurements are summarized in Figure 42. Like after exposure to the 9.3 ppm concentration, VO_{2max} values also became progressively reduced over time after exposure to the higher 13.9 ppm concentration of phosgene. Interestingly, the maximal percentage reduction in VO_{2max} that occurred as of 23 hrs after exposure to 13.9 ppm phosgene was similar to that observed with the lower phosgene concentration. Also like with the 9.3 ppm concentration, the pattern of progressive reductions in work performance capacity following the 13.9 ppm exposures was essentially a mirror image of the progressive increases in LWW values measured after exposure to the concentration of phosgene.

Interestingly, even though VO_{2max} reductions observed with each phosgene concentrations appeared to be associated with the severity of pulmonary edema present at the time of the VO_{2max} measurements, a comparison of the data shown in Figures 41 and 42 suggest that post-phosgene exposure reductions in VO_{2max} may have additional bases that are not depicted by LWW increases alone. Specifically, the ~28% reduction in VO_{2max} following exposure to the 9.3 ppm phosgene concentration was associated with an average

LWW value of ~2.1 g as of 24 hrs after exposure, whereas the the LWW of rats 24 hrs after exposure to the 13.9 ppm concentration was ~2.6 g, and this higher gravimetric value was associated with a slightly less reduction in VO_{2max} of ~26%.

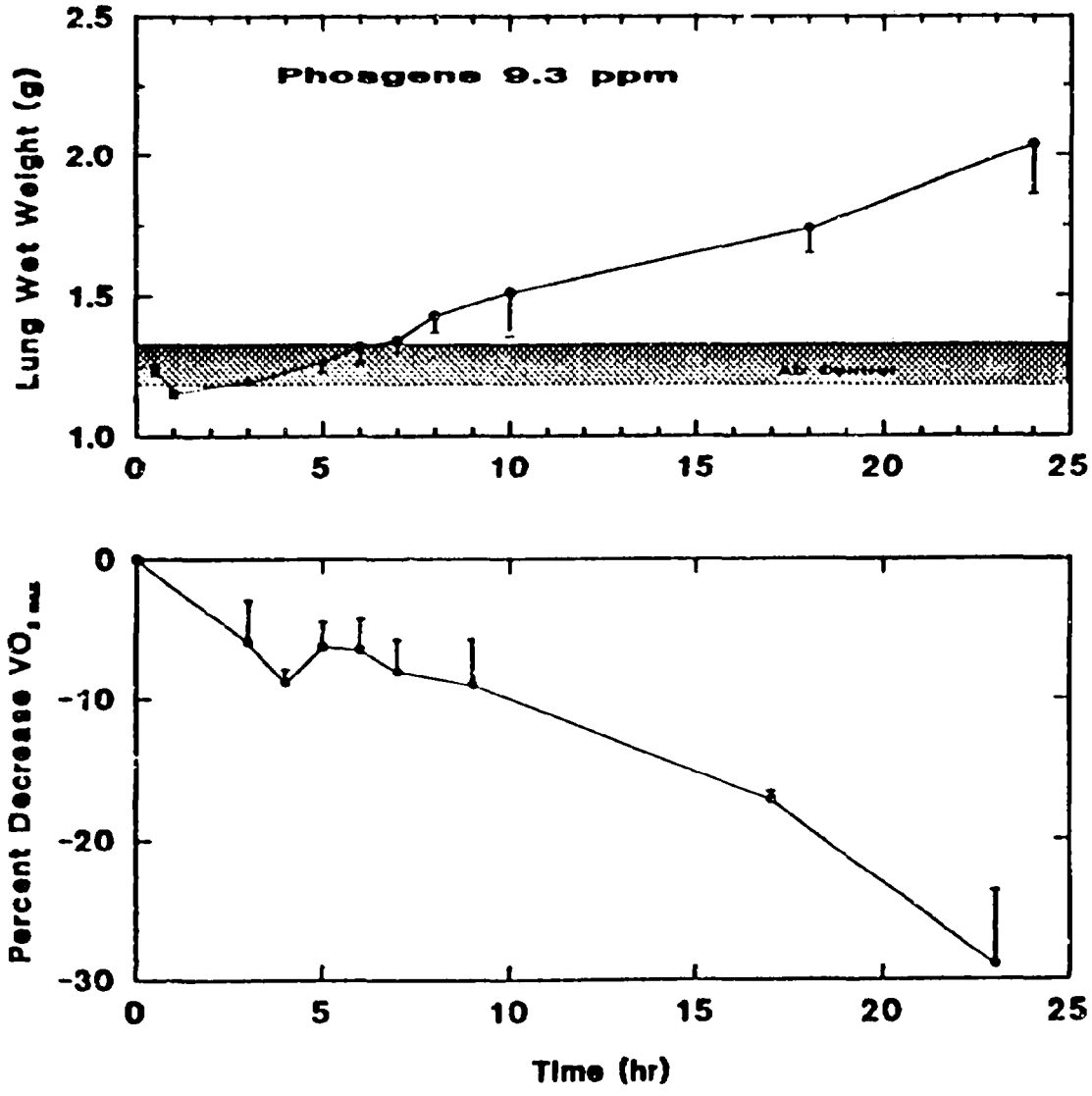

Figure 41 Percent decrease in maximum oxygen consumption ($VO_{2\,max}$) values representing reductions in work performance at various times after 9.3 ppm Phosgene exposure. Also presented are the lung wet weight (LWW) responses of rats exposed to 9.3 ppm Phosgene for 10 minutes. Error bars above the cross-hatch region are significantly greater compared to values measured on air exposed values, $p \leq 0.05$.

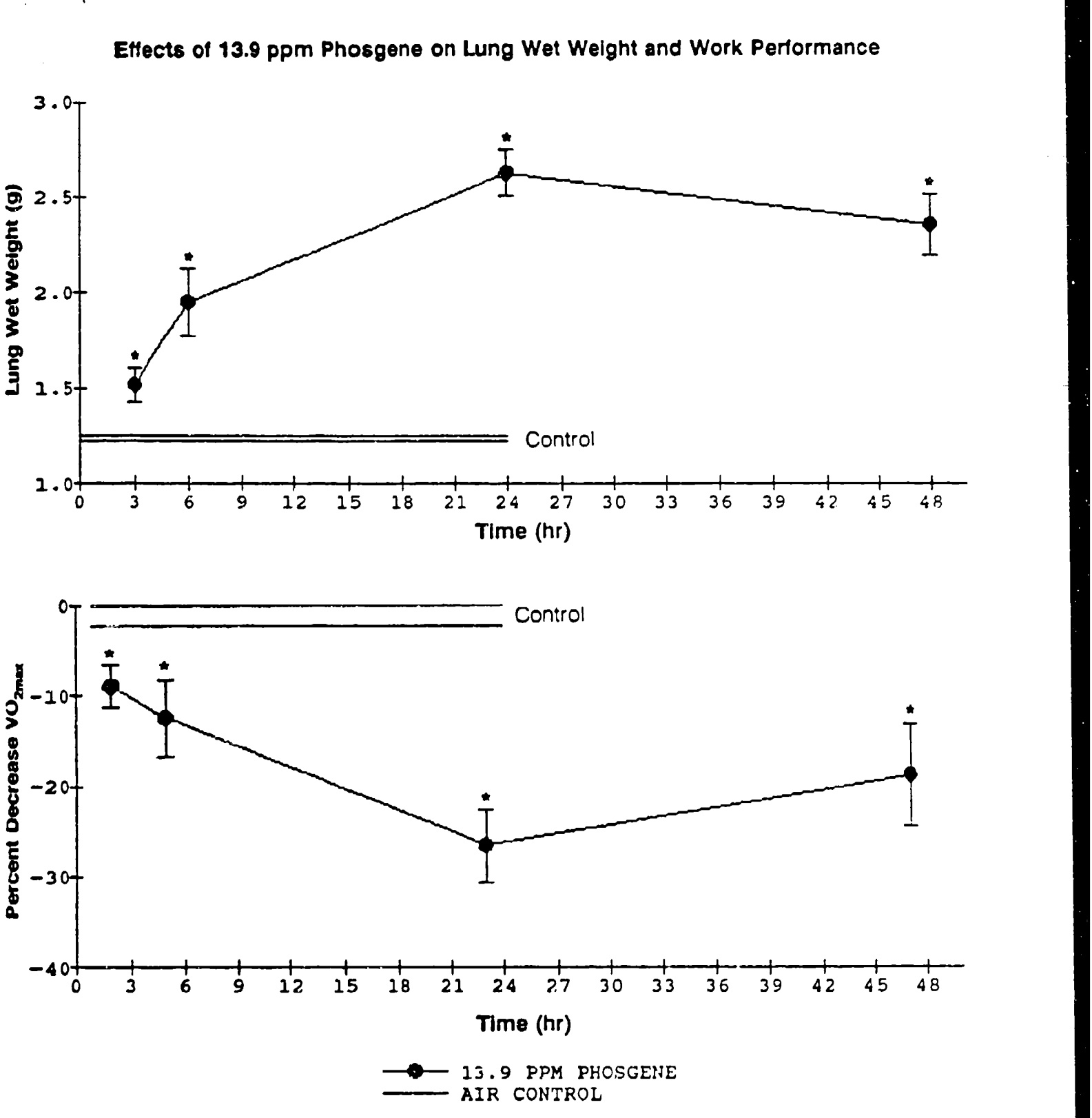

Figure 42 Percent decrease in maximum oxygen consumption (VO_{2max}) values representing reductions in work performance at various times after 13.9 ppm phosgene exposure. Also presented are lung wet weight (LWW) responses of rats exposed to 13.9 ppm phosgene for 10 minutes. Points represent means and standard errors of n=4 or more animals. (*) indicates significant difference from the control ($p \leq 0.05$).

SUMMARY

• An exposure system and exposure monitoring methodology was developed and employed in order to reproducibly expose rats well characterized atmospheres of phosgene.

• Over a concentration range of 9.3 ppm to 32.4 ppm, 10 min exposures to phosgene result in a generally concentration-dependent decrease in rat body weights as of 24 hr after exposure.

• At the altitude of Los Alamos, 32.4 ppm phosgene is the ~LD_{40} for rats (10 min exposure).

• The severity of lung injury (pulmonary edema) that develops as of 24 hrs after phosgene exposure increases with increasing phosgene exposure concentration, but this relationship is not linear.

• Histopathologic changes that occur in the lung following phosgene exposure generally increase with increasing exposure concentration.

• The time to onset of detectable lung injury following phosgene exposure is inversely proportional to exposure concentration, i.e., lower concentrations result in longer "latency periods", higher concentrations result in shorter "latency periods".

• Increases in lavageable protein during latency periods (as defined by the absence of significant lung gravimetric increases) indicate permeability changes can occur in response to phosgene exposure prior to the development of "overt" pulmonary edema.

• Significant increases in lavageable LDH activity during post-exposure latency periods indicate that cellular damage begins shortly after phosgene exposure, i.e., within 1 hr.

• Phosgene exposure can result in significant changes in the numbers and types of lavaged cells. Polymorphonuclear leukocytes in the alveoli are not an early cytological response to phosgene in that increases in these cells do not temporally occur with early increases in lavageable protein and LDH activity. Some evidence was obtained that suggests phosgene may alter the adherence characteristics of alveolar macrophages *in situ*.

• Exposure to phosgene results in decreases in minute ventilation, which are due to reductions in tidal volume and less than compensating increases in breathing frequency

• The co-inhalation of phosgene + 5% CO_2 results in increases in minute ventilation, but such increases are less than that which occur with CO_2 and air.

• Increases in minute ventilation that occur during the co-inhalation of phosgene + CO_2 are associated with a shortening of post-exposure latency periods, and an enhanced severity of the pulmonary edematous response.

• The performance of exercise following the inhalation of phosgene can result in a significant potentiation of the post-exposure pulmonary edematous response. However, at lower phosgene concentrations, i.e., 9.3 ppm, post-exposure exercise generally does not cause a significant potentiating effect even when performed during the course of developing overt pulmonary edema.

• The ability of post-exposure exercise to potentiate the injurious response to phosgene does not appear to be due to an exercise-associated enhancement of cell killing or an enhancement of protein translocations into the alveoli.

• The performance of exercise after exposure to phosgene + CO_2 results in greater potentiation of the injurious response than when exercise is performed following exposure to phosgene only.

• Significant reductions in work performance capacity can follow phosgene exposure. For a given mass concentration, the magnitudes of decreases in VO_{2max} are generally inversely proportional to the extent of the pulmonary edematous response present at the time of the VO_{2max} measurement. Some evidence was obtained that suggests phosgene-induced reductions in VO_{2max} may not exclusively be related to the severity of pre-existing pulmonary edema.

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